PN-ABC-819 61664

Strengthening Collaboration in Biotechnology: International Agricultural Research and the Private Sector

Bureau for Science and Technology
Agency for International Development
Washington, DC 20523

Strengthening Collaboration in Biotechnology:

International Agricultural Research and the Private Sector

Proceedings of a Conference held April 17-21, 1986 in Rosslyn, VA

Bureau for Science and Technology
Office of Agriculture
Agency for International Development
Washington, DC 20523

Library of Congress Catalog Card Number: 88-7875

Printed in the U.S.A.
April 1989

Library of Congress Cataloging in Publication Data

Cohen, Joel I. 1950-
Strengthening collaboration in biotechnology

Bibliography: p. 474
1. Agricultural biotechnology—Research—International Cooperation--Congresses.
S494.5.B563S77 1989 630'.72 88-7875

Contents

Preface v
N. C. Brady

Acknowledgements vi

Steering Committee Members vii

Acronyms ix

Session I. Building Bridges of Collaboration

Strengthening Collaboration in Biotechnology: International Agricultural Research and the Private Sector 1
N. C. Brady

Conference Overview and Technology Integration 7
Joel I. Cohen

Building Bridges of Research Collaboration Through the CGIAR System of Centers 13
Richard L. Sawyer

Research Collaboration and Technology Transfer: The Public and Private Sectors in Developing Countries and the International Seed Companies 21
Donald N. Duvick

Session II. National and Regional Biotechnology Programs: Needs and Opportunities

Biotechnology in Thailand: Present Status, Needs and Opportunities for International Collaboration 33
Yongyuth Yuthavong and Sakarindr Bhumiratana

Biotechnology: New Approach for Agricultural Research and Training at CATIE 53
Victor Villalobos

Session III. Applications of Plant Cell and Tissue Culture for Crop Improvement

Use of Cell Genetics in Crop Improvement 69
Maro Sondahl and David Evans

Tissue Culture Micropropagation and CIAT Biotechnology Research 77
William Roca and B. Nolt

Breeding for Rice Varieties Tolerant to Adverse Conditions Through Tissue Culture at IRRI 93
Francisco J. Zapata and L. B. Torrizo
Tissue Culture for Germplasm Management and Distribution 109
John Dodds
Impact of Somaclonal Variation on Plant Improvement and IARC/Private Sector Collaborative Research 129
William Scrowcroft

Session IV. Commercialization and Research Perspectives for Vaccines
Commercialization and Research Perspectives for Vaccines 141
Charles Muscoplat
Vaccine Development: The Role of Biotechnology, ILRAD and the Private Sector 151
John Doyle

Session V. Molecular Technologies for Crop Improvement: Viral and Pest Management, Molecular Markers, and Recombinant DNA
Genetic Transformation for Virus Resistance: Needs and Opportunities in Developing Countries 157
Roger N. Beachy
Genetically Engineered Insect Resistance in Crops 165
Ronald Meeusen
Application of Restriction Fragment Length Polymorphism to Plant Breeding 175
David P. West, J.H. Cramer, J. Romero-Seversen, Y. Ma, M. Murray
Development and Field Testing of Crops Improved Through Genetic Engineering 185
Xavier Delanny, K.T. Finley, S.G. Rogers, R.B. Horsch, G.M. Hishore, R.N. Beachy, N.N. Tumer, D.A. Fischhoff, H.J. Klee, D.M. Shah

Session VI. Facilitation of Wide Crosses Through Biotechnology
Use of Hormones and Ovule and Embryo Culture to Enhance Wide Crosses in Arachis 197
J. Philip Moss, D.C. Shastri, and L. Aruna
Alien Germplasm for Wheat Improvement Facilitated by Cytogenetic Manipulation and Use of Novel Techniques 211
Abdul Mujeeb-Kazi and Robert Asiedu

Session VII. Genetic Resources: Commercial and Public Initiatives Linking Conservation, Evaluation, and Utilization

International Cooperation on a Major World Crop Genetic Resource: The Latin American Maize Project (LAMP) 233
Quentin Jones, Ricardo Savilla, and Wilfredo Salhuana

Plant Protection In Developing Countries 247
Stanley D. Schlosser

Plant Genetic Resources Network in India 255
Rajendra Singh Paroda

Session VIII. New Agendas for Collaborative Research and Commercialization

Focused Large-Scale Cooperative Research and Commercialization 267
Richard Baldwin

CIMMYT's Initiative In Biotechnology and the Initiation of an International Collaborative Network on Maize Biotechnology Using Molecular Markers—Role of Private Sector 279
Clive James

Session IX. International Funding Agency Support of Biotechnology Research

The Rockefeller Foundation's International Program on Rice Biotechnology 291
Gary Toenniessen and Robert W. Herdt

Biotechnology and Donor Opportunities: Experiences in India 319
Kerri-Ann Jones

Session X. Biosafety Concerns Regarding Agricultural Research

Development of Guidelines and Regulations for Safe Conduct of Agricultural Research 329
Sue Tolin

International Agricultural Research and Biotechnology: Strategies for Safety 341
Anne Hollander

Session XI. Biotechnology-Based Animal and Plant Diagnostic Tests

Crop Disease Detection Through Biotechnology—Worldwide Applications 369
Richard Lankow

Immunoassay Technology: Diagnosis of Livestock Disease and Its Use in Developing Countries 383
Erwin F. Workman, Jr.

Applications of Immuno and DNA Hybridization Diagnostics in Research at ICARDA 399
Khaled Makkouk, Douglas Beck and Marlene Diekmann

Session XII. Commercialization, Patents and Technology Transfer

One Company's Attempt to Commercialize an Agricultural Biotechnology Technology 413
Peter Carlson

A New Era of Government-Industry Cooperation in Research and Development 423
William Tallent

Conference Comments: Biotechnology and World Food Demand 429
Norman E. Borlaug

Workshop Reports.

Biosafety in Agricultural Research 437
Bruce Umminger

Application of Diagnostics for Plant and Animal Pathogens 443
Joyce Turk, Joel Cohen and Donald Lechsinger

Genetic Resources/Wide Crosses 447
Robert Bertram

Molecular Technologies 455
David Edwards

Plant Cell and Tissue Culture 459
Oluf Gamborg

Vaccine Development 461
Michael Burridge

Registration List 465

Preface

The U.S. Agency for International Development is committed to helping people in developing countries improve the condition of their lives. In this process, the importance of productive, sustainable agriculture cannot be overestimated. The new methods of biotechnology are important tools in addressing constraints to sustainable agricultural development. Involvement of the private sector should hasten the process of moving research results from scientists' laboratories to farmers' fields.

A.I.D. sponsored the conference, **Strengthening Collaboration in Biotechnology: International Agricultural Research and the Private Sector,** in order to bring together those working in this field. Our purpose was twofold: first, to provide a forum for discussing the latest applications of biotechnology to crop and livestock improvement and their potential benefits to international research initiatives; and second, to provide the framework for exploring collaborative research and commercial development of agricultural products.

The results of the conference more than met our expectations. The high quality of the presentations and the frank exchange in the discussions set a precedent for future dialogues. The workshops and informal sessions provided opportunities for beginning to build linkages between the international agricultural research system and the private sector in applying biotechnology to the problems impeding agricultural development in the Third World.

This publication of the conference proceedings includes the formal presentations, reports and recommendations from the six conference workshops, and complete registration list. The enthusiastic efforts of individuals who participated in the conception, development, and organization of this conference are sincerely appreciated. I also want to thank all those whose participation made the conference a success.

N. C. Brady
Senior Assistant Administrator
for Science and Technology

Acknowledgements

The publication of these proceedings and the formulation and organization of the conference itself was made possible through the continued support of numerous individuals. First, it should be said that neither the conference nor this report would have been possible without the direction and support provided by Dr. Nyle C. Brady, Senior Assistant Administrator for Science and Technology, U.S. Agency for International Development. Dr. Brady provided the stimulus for the conference and served as Chairman of its Steering Committee.

The members of the Steering Committee provided valuable contributions throughout the planning stage. Their representation from public and private sector institutions, CGIAR centers and regional and central bureaus of A.I.D. provided a working model of joint collaboration. Careful consideration was given by each member to session organization and formal presenters. The Steering Committee members are listed on the next page.

By providing assistance to the Steering Committee, each member of the Conference Planning Committee facilitated the actual outcome of the Conference. The Conference Planning Committee members included Kathy Alison, Veronica Priddy, Linda Spink, Brenda Sharpe, and Bruce Crossan of USDA/OICD and Rob Bertram and Kim Wilkey of A.I.D.

Each session was chaired by individuals internationally known in agricultural research. These included: William Furtick, Max Rives, Indra Vasil, George Shibley, Malcolm Finlayson, J.M.J. de Wet, G.S. Khush, Trevor Williams, Gabrielle Persley, Dilbagh Athwal, John Nickel, and Allen Stevens. Their participation greatly enhanced the Conference program.

Each workshop discussion was facilitated by distinguished researchers committed to each particular topic. The reports were written by a second individual serving as reporter. The facilitators included: T.D. Yilma, Ronald Philips, Rita Colwell, Henry Miller, Donald Plucknett, and Frederick Bliss. The reporters were Michael Burridge, Oluf Gamborg, David Edwards, Bruce Umminger, Robert Bertram, and Joyce Turk.

Finally, A.I.D. would like to acknowledge special support provided by David Bathrick, Head, and Harvey Hortik, Division Chief, of the Office of Agriculture. A.I.D. support was also provided by Marcia Packer, Norma Ayers, Erin McDavid, and Bess Willis.

J.I. Cohen
Office of Agriculture
April, 1989

Strengthening Collaboration in Biotechnology: International Agricultural Research and The Private Sector

STEERING COMMITTEE

CHAIR

N. C. Brady
Senior Assistant Administrator
for Science and Technology
Agency for International Development
Washington, D.C.

MEMBERS

William Brown
Chairman, Board of Agriculture
National Academy of Science
President Emeritus
Pioneer Hi-Bred International
Johnston, Iowa

Peter Carlson
Vice President
Research and Development,
Crop Genetics International
Hanover, Maryland

Richard Cobb
Deputy Director
Office of Technical Resources
Bureau for Asia Near East
Agency for International
Development
Washington, D.C.

Joel Cohen
Program Director and
Editor of Proceedings
Office of Agriculture
Bureau for Science and
Technology
Agency for International
Development
Washington, D.C.

Rita Colwell
Director
Maryland Biotechnology
Institute
University of Maryland
Adelphi, Maryland

David A. Evans
Vice President, Corporate
Research
DNA Plant Technology
Corporation
Cinnaminson, New Jersey

John A. Fulkerson
Principal Scientist
U.S. Department of Agriculture
Cooperative State Research
Service
Washington, D.C.

William Furtick
Agency Director for Food and
Agriculture
Bureau for Science and
Technology
Agency for International
Development
Washington, D.C.

Paul Hughes
Director of Corporate Relations
World Resources Institute
Washington, D.C.

Ronald L. Phillips
Professor
Dept. of Agronomy and Plant Genetics
University of Minnesota
St. Paul, Minnesota

Donald Plucknett
Scientific Advisor,
Consultative Group on International Agricultural Research (CGIAR)
The World Bank
Washington, D.C.

Richard L. Sawyer
Director General
Centro Internacional de la Papa (CIP)
Lima, Peru

Strengthening Collaboration in Biotechnology: International Agricultural Research and The Private Sector

ACRONYM	NAME	HEADQUARTERS
AID	Agency for International Development	Washington, D.C.
AGR	Agriculture/S&T/AID	Washington, D.C.
AP	Agriculture Production/S&T/AID	Washington, D.C.
APHIS	Animal & Plant Health Inspection Service/USDA	Washington, D.C.
ARS	Agricutural Research Service/USDA	Beltsville, MD
AVRDC	Asian Vegetable Research Development Center	Tainan, Taiwan
CATIE	Centro Agronomico Tropical de Investigacion y Ensenanza Center for Tropical Agricultural Research and Training	Turrialba, Costa Rica
CENARGEN	National Center for Genetic Resources	Brazil
CGIAR	Consultative Group on International Agricultural Research	Washington, D.C.
CIAT	Centro Internacional de Agricultura Tropical International Center for Tropical Agriculture	Cali, Columbia
CIMMYT	Centro Internacional de Majoramente de Maiz y Trigo International Maize and Wheat Improvement Center	El Batan, Mexico
CIP	Centro Internacional de la Papa International Potato Center	Lima, Peru
CSRS	Cooperative State Research Service/USDA	Washington, D.C.

FAO	Food and Agriculture Organization of the U.N.	Rome, Italy
FDA	Food and Drug Administration	Washington, D.C.
FHIA	Fundacion Honduran de Investigation Agricola	Honduras
IADB	Inter-American Development Bank	Washington, D.C.
IARC	International Agricultural Research Center(s)	Worldwide
IBPGR	International Board for Plant Genetic Resources	Rome, Italy
ICARDA	International Center for Agricultural Research in Dry Areas	Aleppo, Syria
ICIPE	International Crops Research Institute for the Semi-Arid Tropics	Nairobi, Kenya
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics	Hyderabad, India
IFAR	International Fund for Agricultural Research	Arlington, Virginia
IFPRI	International Food Policy Research Institute	Washington, D.C.
IITA	International Institute of Tropical Agriculture	Ibadan, Nigeria
ILCA	International Livestock Center for Africa	Addis Ababa, Ethiopia
ILRAD	International Laboratory for Research on Animal Diseases	Nairobi, Kenya
INIBAP	International Network for Improvement of Bananas and Plantains	Montpellier Cedex, France

IRRI	International Rice Research Institute	Manila, Philippines
ISC	International Seed Companies	Worldwide
ISNAR	International Service for National Agricultural Research	The Hague, Netherlands
ITD	International Training Division/ OICD/USDA	Washington, D.C.
LAMP	Latin American Maize Project/ ARS/USDA	Beltsville, MD.
NARS	National Agricultural Research Systems	Worldwide
NAS	National Academy of Sciences	Washington, D.C.
OES	Bureau of Oceans & International Environmental and Scientific Affairs/Department of State	Washington, D.C.
ODNRI	Overseas Development Natural Resources Institute	London, G.B.
OICD	Office of International Cooperation & Development/USDA	Washington, D.C.
ORSTOM	L'Office de la Recherche Scientifique et Technique d' Outre-Mer	France
PO	Program Office/AID	Washington, D.C.
RFLP	Restriction Fragment Length Polymorphism	Research
ROKUPR	Regional Mangrove Swamp Rice Research Station	Freetown, Sierra Leone
SAA	Senior Assistant Administrator/AID	Washington, D.C.
S&T	Bureau for Science and Technology/AID	Washington, D.C.

STDB	Science and Technology Board	Bangkok, Thailand
TAD	Technical Assistance Division/ USDA	Washington, D.C.
UN	United Nations	New York, N.Y.
UNDP	United Nations Development Programme	New York, N.Y.
USDA	United States Department of Agriculture	Washington, D.C.
TCARC	Technical Centre for Agriculture and Rural Cooperation	Wageningen, The Netherlands
WARDA	West Africa Rice Development Association	Monrovia, Liberia
WB	World Bank	Washington, D.C.

Strengthening Collaboration in Biotechnology: International Agricultural Research and the Private Sector

N.C. Brady
Senior Assistant Administrator for Science and Technology
U.S. Agency for International Development
Washington, DC

On behalf of the Agency for International Development, I want to welcome you to this International Biotechnology Conference, the first of its kind to be sponsored by A.I.D. Our mutual interest in both research and development assistance provides an excellent foundation for the discussions that will take place here during the next several days.

As representatives of the international development, research, and private sector communities, we will:

- share our perspectives on the state-of-the-art in biotechnology and its applications in the Third World;
- speculate on future developments in this important emerging field; and
- begin a linkage process to ensure that developing countries will benefit from biotechnological research as its applications continue to increase in number and variety.

The Necessary Role of New Technology in Development

The necessity of new and improved technologies to support development assistance has been debated again and again during the last three decades. In the early years, development experts assumed that the necessary technologies existed in the industrial world and just needed to be transferred to newly independent developing countries. Research endeavors during and after World War II produced technologies that were widely adopted in developing countries. These included, for example, antibiotics, the smallpox vaccine, and malaria controls.

Some of the medical breakthroughs have been extremely valuable, especially in countries where the physical and human channels exist to deliver effective, affordable drugs, vaccines, and other treatments. The evolution of contraceptive technology, which began in the 1960s, continues to generate a more responsible view of human reproduction, and has, in that process, given women much

more control over their reproductive potential. Family planning is now a widely accepted concept that will eventually have almost universal application in one form or another.

In many cases, however, the desired results could not be accomplished with existing technologies. Further research was needed, at the very least, to adapt existing technologies to local conditions so that effective application could be achieved. Nowhere was the need for research more evident than in agriculture, where rainfall, temperature, soil quality, and numerous other variables usually preclude direct transfer of even the most promising technologies. Only through research carried out in the countries themselves could scientists generate or adapt the new technologies needed to overcome agricultural constraints, increase agricultural productivity, and move economic development forward.

In the 1960s and early 1970s, the pressures of overpopulation and hunger precipitated in-country agricultural research that led to improved varieties and management packages. Subsequent adaptation made these new technologies effective in large areas of the developing world, particularly in Asia and Latin America. The resulting Green Revolution greatly increased wheat and rice production worldwide.

For many of the world's people, unfortunately, these scientific achievements have made very little difference. The people who remain virtually untouched by technological advances live in the least-hospitable, least-accessible rural areas of the developing world. Many of them have meager, undependable incomes and poor access to energy resources. Even where significant progress has taken place, more research is needed as population, and resulting pressure on the productive resource base, increases. For many poor people in the developing countries, the simple expectations of reasonably good health, sufficient food, and a predictable, dependable income are still only a dream.

Support of scientific research that yields more-suitable technologies is one way to give substance to those not-very-ambitious expectations. As we break down the barriers to knowledge, and increase our ability to manage and sustain the natural environment, we come closer to solving the more tenacious problems that still plague the lives of so many people in the developing world.

Biotechnology and Development Assistance

Today, we are entering an era of unprecedented scientific potential in which biotechnology is already playing a major role. The new

technologies have opened new ways to attack problems and constraints, and research efforts are finding new applications daily. Unless we also address Third World problems in that effort, the already enormous chasm that exists between developed and developing countries will grow wider. Apart from basic humanitarian considerations, there are sound economic reasons for using these "state-of-the-art" approaches to solve LDC problems. LDCs have more to gain than developed countries in terms of overcoming diseases and insects, and soil toxicities.

What A.I.D. is Doing

Understanding the need and potential that exists, A.I.D. has begun a variety of programs to harness biotechnology to address development problems. In agriculture, researchers under one project are designing a better vaccine for rinderpest, an acute, highly contagious viral disease of ruminants. Another group is attempting to increase the efficiency of nitrogen-fixing organisms, and their production, distribution, and use in Third World settings. Other AID-supported scientists are helping developing countries establish capability in plant tissue-culture research to overcome toxic-soil constraints to increased crop production.

Many of the international agricultural research centers (IARCs), to which A.I.D. provides core budget support, as well as several of A.I.D.'s Collaborative Research Support Programs (CRSPs) are using various biotechnological methods to speed the development and testing of plant varieties with tolerance to adverse soil, moisture, and temperature conditions. Our Science and Technology Initiative (STI) with India is employing biotechnology in several joint research efforts.

Clearly, we view biotechnology as an integral and valuable tool in our agricultural research arsenal—not as an isolated, esoteric endeavor. A.I.D. is in the business of dealing with real problems by identifying and developing real solutions. That focus is exemplified in our agricultural research endeavors, including use of the new technology. Molecular biology holds out a promise we cannot ignore. We know that if the products of biotechnological research are to become widely available in the developing countries, there needs to be movement from the scientist's laboratory and test plots to the fields of farmers in developing countries. We are seeking your collaboration in this effort through wide-ranging formal contacts and through informal contacts stimulated at a meeting such as this. We are hopeful that the collaborative approach which characterizes the U.S. private sector's relationship with U.S. universities and other research institutions can be duplicated in the international arena to the benefit of the developing countries.

Involvement of the Private Sector

Thus, one of the Agency's major strategies to meet development goals is increased involvement of the private sector, both in this country and the developing world. U.S. private enterprise is already at the forefront of biotechnological research and commercialization. In addition to a large and capable scientific community, an aggressive and savvy business community has welcomed high-risk, high-potential ventures associated with research. In the biotechnology arena, the private sector has especially good potential for active involvement as we move needed LDC technologies from government-funded basic research to private sector development of commercially viable applications.

Issues Unique to Biotechnology

The involvement of private firms and laboratories in research and technology applications has been valuable, and is increasing. But collaboration on research involving biotechnology presents difficult issues in developing countries, similar to those that have already surfaced in the United States. These issues must be resolved if the developing world is to have access to the benefits of this new agricultural research. In fact, a root cause of some of these problems is the speed with which applied research in this field can produce patentable products with a high market value.

The issue that has become increasingly important in the United States, and soon will in the developing countries also, is one of health and safety to people and protection of the environment. While this issue is still being debated in the popular press as well as in scientific journals, we at A.I.D. must exercise due caution and international responsibility for biotechnology applications we foster. A newly created Standing Committee on Biotechnology is responsible for monitoring A.I.D. projects and activities in this domain. The committee's formal working group has identified three initial goals:

1. to inventory all A.I.D. projects that involve biotechnology research or products;

2. to develop a review process for Agency biotechnology projects; and

3. to educate appropriate A.I.D. staff about general biotechnology techniques and applications.

We are also involved in the interagency councils founded specifically to address the special issues related to biotechnology.

Another issue is, of course, one that is commonly referred to as the "intellectual property rights" or "technology drain" issue. Here, I believe that a number of well-thought-out procedures can help to facilitate successful cooperation.

First, I believe there needs to be a framework within which scientists can collaborate without concern about proprietary risk. Researchers in the private sectors and national research systems of industrial and developing countries who work with counterparts in the international research centers must be assured that the fruits of their labors will not be improperly appropriated. Developing country researchers, particularly, are sensitive to the use of their "intellectual property" to profit the industrialized countries with little benefit to their own.

Second, through positive, productive interactions, developing countries must be helped to overcome their negative perceptions of the industrialized world private sector.

Third, developing country scientists and governments must be assured that applications intended for their specific adaptation and use can be safely tested and applied in their countries, some of which have a more fragile natural resource base than do the industrialized nations.

To these ends, generic questions about regulation, patent rights, biosafety, and so forth will be openly addressed in the final sessions of this conference. Hopefully, this will be the beginning of not only useful dialogue, but also fruitful cooperation among the groups represented here today.

As collaboration increases, we expect that the scientific capacities of developing countries will be strengthened to work with recombinant DNA, cell fusion, and other bioprocessing techniques. The cadre of scientists thus trained will become the backbone of developing country efforts to meet the agricultural production challenges they face today. Human resource development in this emerging field will require very careful guidance.

This conference provides a very timely forum in which to explore the issues of biotechnology cooperation and ways in which they can be resolved. You collectively represent an ideal combination to do that and to identify areas of potential specific collaboration. Among you are scientists and research leaders from public and private institutions from both developed and developing countries; corporate leaders faced with major investment decisions on a day-to-day basis; and policymakers from governments and international

agencies responsible for deployment of public resources. Most important, cutting across all the groups present, there is also considerable general experience in what makes development work.

This, then, is a very special opportunity. I look forward to a "wrap-up" session on Thursday that distills your discussions and summarizes your insights and recommendations. We all want to move ahead to harness these exciting new development tools that will help us reach the longstanding goals —to eradicate hunger, hasten economic development, and bring prosperity to people everywhere.

Conference Overview and Technology Integration

Joel I. Cohen
Office of Agriculture
Agency for International Development
Washington, DC

I would like to extend my welcome to everyone here as well as my thanks to the Program and Steering Committee members who have helped develop, guide and implement this Conference. Members of both committees, those due special thanks and a complete list of presenters is found immediately after the Program.

Technology Integration

Biotechnology encompasses an array of techniques providing new abilities to manipulate germplasm. While much attention has focused on potential achievements that biotechnology offers for transferring genes between organisms, relatively little attention has focused on institutional arrangements that enhance the integration of biotechnology in agricultural research. Purposeful integration of biotechnology with conventional crop improvement programs, as opposed to this integration occurring on its own—or not occurring at all—increases opportunities to maximize efficiency of available funding, personnel, and facilities while accelerating delivery cultivars to farmers.

As detailed by Dr. Brady, our mutual goal is to identify, explore, and develop new linkages for collaborative research and/or commercial product development. While the Conference has focused on representative technologies, it is not intended to dissociate these from traditional or conventional approaches. Rather, to identify research objectives that completely integrate cellular and molecular technologies with conventional crop (Table 1) and livestock improvement. The technologies which we have identified have been grouped into five sessions, including the following: plant cell and tissue culture, vaccine development, molecular technologies (including genetic engineering and restriction fragment length polymorphism), and immunodiagnostics.

Table 1. Potential applications of biotechnology for integration with conventional cultivar development.

COMPONENTS OF CONVENTIONAL GERMPLASM-BASED TECHNOLOGIES	CONVENTIONAL TIME SPAN	POTENTIAL BIOTECHNOLOGY CONTRIBUTIONS
I. Genetic Resources		
A. Acquisition/Exchange	1 year	*In vitro* culture, disease indexing and eradication, micropropagation
B. Conservation	Ongoing	*In vitro* conservation, gene libraries
C. Evaluation	2 seasons	Molecular diagnostics, RFLPs
D. Germplasm Enhancement	3-5 seasons	Embryo rescue, molecular diagnostics, selection in tissue culture, somaclonal variation, gene transfer
E. Wide Hybridization	2 years	Embryo rescue, somaclonal variation, anther culture, protoplast fusion
II. Breeding		
A. Selection of Parental Germplasm 1. Elite lines 2. Adapted populations 3. Exotic materials		Molecular diagnostics, tissue culture derived lines, gene transfer
B. Initial development cross (F1)	1 year	
C. Production & Selection of Segregating lines (F2-F3)	2 seasons	Somaclonal variation, anther culture, molecular diagnostics
D. Controlled inbreeding (F4-F7)	3-4 seasons	
E. Bulk increase of finished lines	2 seasons	Pathogen elimination, micropropagation
III. Testing		
A. Observational Trials and/or Preliminary Testing	2 seasons	
B. International trials	2 seasons	Molecular diagnostics
C. Advanced testing in national coordinated trials	2 seasons	
D. Farmer's field trials	2 seasons	
IV. Distribution		
A. Bulk increase	1-2 seasons	Micropropagation
B. Certification	1 season	Disease indexing and eradication
C. Quarantine	1 season	Disease indexing, molecular diagnostics, micropropagation

Biotechnology Integration at International Agricultural Research Centers

The goal of biotechnology at the IARCs is to enhance the efficiency of established breeding programs and to devise new crop improvement strategies as quickly as possible. These centers are assuming an important role in identifying appropriate biotechnology applications in agriculture, training scientists from national programs, and transferring technologies to national programs so that their expertise does not lag behind that of industrialized nations and the private sector.

IRRI is developing its biotechnology program for rice through efforts begun within its Plant Pathology Department in 1981. Now contained within the Innovative Breeding Technologies Unit (IBT)—in turn, a part of the Germplasm Evaluation and Utilization program—IRRI's biotechnology program is heavily involved in anther culture research. The original objectives of the anther culture program were to establish effective regeneration and plating methodologies and to determine which rice varieties were capable of regeneration. More recently, specific programs have been established to support conventional rice breeding efforts, such as rapid production of homozygous lines and increasing selection efficiency of desirable traits.

The IBT unit involves a interdisciplinary team of five scientists. A plant physiologist heads the anther culture effort. The IBT is also involved in tissue culture, wide hybridization, and hybrid rice. Expanded biotechnology programs generate additional germplasm and lines which require breeding, testing, and storage. To accommodate this material, the IBT unit recently added a facility to house working collections of rice lines derived from tissue culture.

CIAT established its Biotechnology Research Unit (BRU) for work on cassava, rice and beans in 1985. The BRU emphasizes applied biotechnology and serves as a liaison between CIAT breeders and institutions that develop technologies of potential value for germplasm improvement. Current research areas include haploid production in rice and beans, embryo rescue in interspecific bean crosses, and collaborative research on gene transfer and recombinant DNA.

ICRISAT scientists within the legume program employ embryo rescue to sustain wide crosses. A unified biotechnology program is being proposed at ICRISAT that would include embryo rescue, haploid production in peanut and cereals, pollen transformation in sorghum and pearl millet, construction of a restriction fragment map for sorghum, and molecular diagnostics. Such efforts will con-

tribute to conventional breeding efforts through identification and transfer of genes contributing to agronomic fitness.

Collaborative potato research at CIP has extended capabilities in biotechnology to include genetic engineering, protoplast fusion, and anther culture. A synthetic gene which enhances nutritional value has been inserted and expressed. However, appropriate regulations will need to be established before these plants can be field tested. Protoplast fusion is facilitating the transfer of cytoplasmic male sterility. Anther culture research seeks to understand mechanisms responsible for obtaining dihaploids from diverse genetypes.

Conference Structure

Identification of integrated, collaborative research opportunities is not a function of technologies alone, but also the national and international context in which they will be developed, tested and utilized. Because of this fact, the Conference has been divided equally between technology-oriented and contextually-oriented sessions, each one listed in the Program section of your Conference notebook. To facilitate identification and discussion of collaborative opportunities, specific topics of common interest will be covered in each talk:

1. First, each speaker will present the current "state-of-the-art" of their respective subject, whether that be a technology or contextual issue.

2. Second, they will discuss anticipated problems and needs, and,

3. Finally, present potential areas for joint collaboration, development, and/or commercialization, if appropriate.

Each of the 13 plenary sessions is scheduled for 90 minutes with 20-30 minutes reserved for discussion. Please hold your questions until the presenters have finished their talks, at which time the Session Chair will moderate the resultant discussion.

Formal presentations are directly related to six workshops addressing the following topics: vaccines, cell and tissue culture, molecular technologies, biosafety, genetic resources, and diagnostic techniques. The first three are on Tuesday afternoon from 1:30-3:30 and the final three on Thursday morning from 10:30-12:30. These workshops provide an opportunity to formalize key recommendations concerning collaborative research initiatives. This is your opportunity to enhance the meeting's value by presenting unique perspectives not able to be included in the formal program.

The workshop discussions will register the interest in specific technologies and their utility to scientists in developing countries. They will also indicate abilities and interests in commercialization and suggest strategies which ensure safe product development and testing. Finally, they offer each of us the chance to consider the opportunities and concerns regarding relevant technologies and to use this information in providing guidance to our respective institutions.

As this is an international forum, we want to particularly encourage our distinguished international colleagues to use this opportunity to inform the United States agricultural and development community as to their perception and interests in collaborative research. Of particular interest are your perceptions concerning genetic resources, mutual benefits of patent protection, biosafety, ability to support collaborative and commercial research, and national strategies in biotechnology. These topics, and others, will be covered formally by representatives from CIP, Mahidol University of Thailand, CATIE, CIAT, IRRI, ILRAD, ICRISAT, CIMMYT, National Bureau For Plant Genetic Resources Of India and ICARDA.

On the domestic side, we would hope that the representatives from agricultural biotechnology and seed companies, and universities, will be willing and interested in forming research partnerships with scientists from the international community. Your concerns of proprietary rights, market assessment, product development, adequate and safe testing arrangements should be brought to the forefront. These topics, as well as proprietary technologies, will be covered formally by representatives from Pioneer Hi-Bred International, DNA Plant Technology Corporation, Molecular Genetics Inc., Biotechnica Canada, Rohm and Haas, Agrigenetics Advanced Science Company, Monsanto, Agri-Diagnostics Associates, AgriTech Systems, and Crop Genetics International.

We look forward to this sharing of perspectives as the first step in the long process required to develop collaborative research which meets the combined needs of all partners. New Agendas in Collaborative Research is the focus of Session 9. Examples of current opportunities illustrative of these concepts are presented by Richard Baldwin and Clive James. Both the complexities and opportunities available to donor organizations with interests in biotechnology will be explored in Session 10 by Drs. Toenniessen and Jones of the RF & USAID, respectively. Special issues pertaining to Plant Genetic Resources will be discussed in Session 8 on Tuesday evening from 7-8:30 P.M.

I would encourage you to take full advantage of our roundtable seating arrangement, coffee breaks, lunch periods and workshops to

meet presenters as soon as possible. Previous commitments and other responsibilities prevents many of our distinguished guests from attending the entire meeting.

We do plan to develop the presentations, workshop dialogue, and conference recommendations into final published proceedings. We hope to move on this ASAP and will be counting on the cooperation of all of our speakers as well as the recording services which we have employed for the conference. Please do not let the tape recording inhibit your questions or discussion and speak up loudly so all can hear.

Please note the reception on Wednesday evening hosted by Administrator Woods and Frank Press, President of the National Academy of Sciences (N.A.S.) at the N.A.S. from 6-7:30.

The CGIAR Centers-Building Bridges of Collaboration Through Biotechnology

Richard L. Sawyer
Director General
CIP
Lima, Peru

Major shifts in agricultural research have taken place during the past 30 years. The importance of public and private sector institutions is changing rapidly at the present time. This is partly due to the limitations of funding for agricultural research from public funds, and partly due to opportunities seen by the private sector for investments that offer a high potential profit. The need to solve food problems in the developing world led to the emergence of a system of international agricultural research centers that is funded through, or associated with, the Consultative Group for International Agricultural Research. It has also led to the creation of programs linking public sector institutions in industrialized countries to programs addressing the food problems of the developing nations.

The role of institutions available for solving agricultural research problems varies from country to country. Although the international agricultural research centers were created to help developing nations, they also have a tremendous importance and payoff for the industrialized nations that fund them. Most developing countries are not yet ready for the major shifts in responsibility for agricultural research from the public to the private sector, which is taking place today in developed countries. Creation and emergence of the new tools of biotechnology such as genetic engineering is providing a golden opportunity for making major progress in solving problems of food, population and the environment. The opportunities and the challenges are so great that a team approach is necessary which will utilize the comparative advantage of each of the research institutions. The challenges in biotechnology today should lead to a global approach to agricultural research involving both the public and private sectors in both developed and developing nations.

CGIAR System

The Consultative Group for International Agricultural Research (CGIAR) was founded in 1971 with centers first funded in 1972. The system has grown quickly to encompass all of the major food crops of the developing world. The 13 centers of the CGIAR and associate centers were established over the past 30 years when

many changes were taking place across the developing world. There is considerable variation amongst the centers, with early centers coming off of foundation drawing boards and later centers coming from various CGIAR approaches and the specific interests of groups of donors or a specific donor.

The gains of some of the early centers led to the much-publicized Green Revolution and high expectations about what CGIAR centers could accomplish. The centers have some excellent capabilities for addressing a portion of the problems of food production for the developing world and at the same time have a real value to the research programs of the developed world. The centers also have some serious limitations that must be recognized as their role in a global agricultural research structure is identified.

For most of the international centers, the collection, classification, maintenance, and distribution of genetic material for the crops within their mandate is a major comparative advantage of value to agriculture in developed and developing countries for public and private institutions. Centers have the continuity and levels of funding to develop and maintain germplasm banks in a way that few national programs can.

Generally, food commodities can be collected, maintained, and exploited most cost-effectively in their areas of origin. Many of the major food crops originated in developing country areas of the world where most of the international centers are located.

The scope of center programs is restricted for a number of reasons. Each of the centers is staffed at about the level of a major university department in the developed world. Thus, programs have been limited to the highest priorities. With the many problems of food production across the developing world, a major portion of the research activities have had to be of a very practical nature. All of the major commodity-oriented CGIAR centers are located in developing countries where servicing and maintenance of the sophisticated equipment now available for biotechnology may be difficult.

CIP Strategies

For these and many other reasons, the success of the CGIAR system is dependent on the quality and quantity of collaborative bridges across the developed and developing world with both public and private sector institutions. Let me use CIP as an example of the kinds of collaborative bridges that most centers have in place today which are valuable for approaching modern biotechnology.

1. Regional Programs

Most centers have global or major regional mandates that require regionalization for working with national programs, transfer of technology, and training. CIP has 8 regional headquarters around the world staffed with senior international scientists and adequate supporting staff who work closely with and support national programs in the area with research, genetic materials, consultants, and training.

2. Country Research Networks

For many small countries, networking is the only way to provide up-to-date access to information, training, and research for priority problems. Some networks are for the sharing of information, others are an actual pooling of research capabilities and depend on each other for priority needs in research, training, and transfer of technology. CIP has five country research networks in place, bridges of research collaboration, some of them active for over 10 years.

3. Research Contracts and Collaborative Programs

The problems across the developing world are many. Some countries now are starting to build the institutions needed to solve food system problems, the kinds of institutions that made the tremendous gains in agriculture across North America and Europe and a few developing countries this past half century. To address the many problems and yet keep the international center programs limited to those areas of research for which they have a comparative advantage, most centers have established bridges with cells of expertise in institutions around the world. CIP has approximately 40 research contracts and a similar number of collaborative projects in place at any given time, and some of these contracts have been active for over 15 years. Thus, there is not only productivity but continuity.

Let me mention briefly some of our *bridges to public sector institutions*. Of the 176 wild tuber-bearing species of potatoes, only 12 have been utilized to any extent for the development of potato varieties around the world up until now. To help us exploit what is maintained in CIP's world potato collection for the future needs of mankind, we fund a number of research contracts in institutions in North America and Europe to help us identify and make available to breeding programs the resistances needed to pests, diseases, and environmental stresses across the world today where potatoes are grown.

Biotechnology plays an important role in all centers maintaining germplasm collections for the development of improved varieties today or for future generations. What used to be annual multiplications in the field with exposure to disease organisms now have become a routine *in vitro* maintenance in a disease-free environment. Thus, the breeder can start off with relatively disease-free parental material, and the cost of maintaining collections can be reduced.

An enzyme-linked immunosorbent assay, ELISA, has been in use at CIP for over 10 years. Through CIP collaboration, this sensitive rapid assay to aid in the elimination of potato viruses and some bacterial diseases is now in use in a number of developing countries.

Potato spindle tuber viroid has been a serious problem or threat to many potato production areas around the world. CIP and USDA scientists at Beltsville, through collaboration, developed a nucleic acid spot hybridization test (NASH) to help eliminate the threat of spindle tuber viroid. The simple part of the process, the extraction of plant sap and spotting onto a test filter, is done in the field by national scientists, whereas the hybridization of the viroid with a radioactive nucleic acid probe is done at CIP headquarters.

The ability to regenerate haploid plants from pollen grains is one of the success stories of plant tissue culture. In collaboration with the Italian Nuclear Research Agency, CIP has explored the reasons why in some potato genotypes it is easy to obtain dihaploids, while in others it seems impossible. So-called "tissue culture ability" has been observed under genetic control, and present data indicate that it might be possible to transfer this characteristic from one genotype to another.

Potato protein has been improved through genetic engineering in collaboration with Louisiana State University. A synthetic gene that produces high-quality protein has been incorporated into the potato genome using agrobacterium as the gene vector. It has been demonstrated that the gene is transcribed and produces a corresponding messenger RNA molecule which is then translated in the plant to produce the synthetic protein.

In collaboration with the Weizman Institution in Israel, CIP is developing a potato protoplast fusion program to transfer nuclei of one genotype to the cytoplasm of another genotype. This technique has already proven useful with some species. Fusion of protoplasts between a male sterile and nonmale sterile potato line should allow us to produce female lines for production of true seed with

male sterility, thus eliminating the need for emasculation of female plants.

In collaboration with Rothamsted Experimental Station in the United Kingdom, CIP is investigating the genetic stability of potato plantlets propagated and regenerated through a wide range of *in vitro* methods to determine levels of somatic mutation in tissue culture material.

In collaboration with the University of Wisconsin, CIP is developing a new *in vitro* methodology of thermotherapy and chemotherapy to clean plants already in an *in vitro* germplasm collection, but still contaminated with viruses. The results from Wisconsin indicate high efficiency. This new methodology would increase the amount of material that can be treated for virus eradication.

The implications of genetic engineering go far beyond the improvement of potato protein quality discussed earlier; it may help to improve resistance to several important pests and diseases. Through additional collaborative links, CIP hopes to genetically engineer potato plants for resistance to viroid and virus infection, by inserting gene sequences that interfere with the replication of these organisms. There may be possibilities in the future of engineering resistance to bacterial diseases such as bacterial wilt and soft rot. Theoretically, the genetic engineering approach may permit production of plants with increased resistance to biological stresses without making major changes in existing agronomically acceptable characters.

4. Bridges to the Private Sector

With the rapid movement of fast-food industry into the developing world, major food processors need local potato varieties that will grow well and provide the accepted standard of processed quality in warm tropical areas. Otherwise, the fast-food industry has to depend on imported frozen products, that elevate the cost of the fast-food service and do not provide the income needed by the resource-poor farmers of the tropics. Through a collaborative arrangement with some major food processors, we are helping develop potential varieties that will grow well in the warm tropics and meet rigid quality standards of the fast-food industry. Obviously, this work is in close collaboration with scientists in national programs where the final testing is conducted and varieties released.

Many of the farmers across the Third World have limited or no resources to control the pests and diseases encountered in food production. Genetic resistance and biological control are necessary low-cost methods of control which have to be explored and utilized wherever possible. All of the commodity-oriented centers give major attention to genetic resistance and biological control of the pests and diseases of their food crops. CIP has identified excellent biological controls for root knot nematodes. However, we are not in the business of producing, packaging and marketing such products. We are in the business of making sure that such products are made available by the private sector at as low a cost as possible to the developing country farmers. Thus, we have a collaborative arrangement with a multinational who is exploring the potential of marketing some of these products, with CIP maintaining the necessary controls to ensure as low a cost as possible in the developing world.

Through these bridges of collaboration, we become very diversified. But this is diversification that leads to strength, not weakness. These bridges have become the strength of CIP, and the strength and success of the CGIAR centers is dependent on such bridges across the system.

Toward a Global System

These bridges of collaboration are becoming invaluable for all of us involved in agricultural research as the new tools of biotechnology become available. And the time is right for all of us to be thinking of a global approach to agricultural research. Through team approaches and networking into the private sector, major progress can be made that would be impossible for single institutions to accomplish in either the public or private sector. The centers can become the bridges from developed to developing countries so that the new tools of biotechnology do not pass by their needed application.

Developed country institutions in the public and private sectors have the kinds of facilities and staff for the evolvement of the tools of biotechnology, whereas the international centers have the kinds of staff and facilities and bridges for the practical application of the tools across the developing world.

Conclusion

This overview indicates that biotechnology is already helping achieve institutional goals within the CGIAR centers and will continue to play an important role. Two major issues will continue to dominate our thinking on the use of biotechnology: (1) Which of the rapidly developing new approaches should be attempted and which might be fruitless? (2) How can we finance new approaches that often require large investments? Collaboration is the key to both issues. The effectiveness of collaborative research with other institutions has already been demonstrated. These linkages with experts worldwide have been extremely important in helping us to pursue the most appropriate available experimental approaches; they will continue to be effective for CIP because they involve institutions that are already making the large financial commitments in personnel and equipment so often necessary to make substantive biotechnological progress. Through collaboration, centers can maintain a "window" on advances in biotechnology that might be useful in solving practical problems for agriculture in developing countries.

Although I have utilized potato and sweet potato examples, other centers are maintaining similar windows on advances in biotechnology that apply to their commodities. Collaborative research is essential if the potential now apparent through biotechnology is to be realized for helping solve the problems of food, nutrition, and environment in the world. Through the bridges of collaboration in biotechnology, we can help establish a global research approach to the major problems confronting agriculture in a way that utilizes comparative advantages of each component.

May this workshop on biotechnology be successful in forwarding a global approach to research through bridges of collaboration across the public and private sectors in the developed and developing world. May these bridges of collaboration in biotechnology lead to improved yields per unit area which we have long been seeking, but also may they lead to improved yields per unit input about which we have been concerned too little up until now. May these bridges of collaboration increase stability and sustainability to agriculture with environmental enhancement and still increase net incomes to farmers with small landholdings across the developing world.

Research Collaboration and Technology Transfer: The Public and Private Sectors in Developing Countries and the International Seed Companies

Donald N. Duvick
Senior Vice President, Research
Pioneer Hi-Bred International Inc.
Des Moines, Iowa

Introduction

Developing countries of the world have as a primary goal the improvement of their food-production capabilities. In general, they need economically and efficiently to increase production per unit of land as well as to expand the land areas suitable for agricultural production. All technologies and all agricultural systems that can increase productivity per unit of input, therefore, are of interest to developing countries. Biotechnology, hailed for the past ten years as uniquely able to give large increases to agricultural productivity, is looked upon with particular interest. Biotechnology sometimes is viewed as a means to bypass, or traverse in shortened time, certain stages in agricultural development. Developing countries are concerned also that delay in adopting this newest aid to agricultural production might put them even further behind the industrial nations in agricultural production capabilities.

I will speak to these concerns from my position in agribusiness, an employee of an international seed company. I speak only for myself, but it is probable that my views in many points will coincide with those of international seed companies based in the industrial nations.

A preliminary comment: international agribusinesses, the "transnationals," are distrusted by some sectors in the developing countries as likely to serve business needs for profits at the expense of the people needs of the developing countries. I will not take up this argument in this forum, but will state simply that I do not think needs of the seed companies for profits are incompatible with their ability to serve some of the true agricultural needs of the developing countries. I will point out, in this discussion, areas in which mutually profitable collaborations could be effected.

International seed companies planning to sell improved seeds in developing countries will find that they have been preceded in most countries by national (public) breeding and seed-distribution organizations, as well as by the international (public) agricultural

research centers, CGIAR-supported organizations like IRRI, CIMMYT, and ICRISAT. The commercial seed firms, if they are to play a useful role in these countries, therefore, must find ways to breed superior varieties or varieties for unfilled niches, and distribute these varieties in needed new ways or in better condition.

One also should note that the absence of breeders' rights (plant variety protection) laws in most developing countries means that seed firms' activities in those countries usually will be restricted to research, production, and sale of hybrid crops such as maize, sorghum, and sunflower. Because self-pollinated crops can be increased and sold by anyone in the absence of legal prohibitions, investment of private funds to breed and sell such crops will not provide acceptable return on investment. But the inbred parents of hybrid crops can be protected physically, and in effect give a kind of intellectual property protection (specifically, trade-secret protection) to their owners.

Complementary Strengths of the Public and Private Sectors

Recognizing areas of potential competition, the private and public sectors then can proceed to explore areas in which they complement each other. (Of course, in many developing countries the need for improved varieties is so great and distribution channels are so undeveloped that there probably will not be much competition between private and public seed supply services for some time to come; products of both sectors will be in demand, to the extent that they meet real needs.)

The greatest strength of international seed companies (ISCs) is in the application (technology transfer) stages of breeding and research: hybrid and variety development and testing, seed production, and distribution.

The international agricultural research centers (IARCs) typically will have greater capability than ISCs in development of basic breeding stocks and in the basic research needed to support applied breeding. IARCs have capabilities in biotechnology research as well, although some ISCs also have large capability in the new sciences that support biotechnology. The national agricultural research systems (NARS) vary greatly in strengths and capabilities. In general, they are strongest in variety development, but now they are interested also in increasing their capabilities in more basic types of research such as cell biology and molecular genetics. They often are greatly restricted in such efforts, however, because of lack of critical mass of skilled scientists per country, as well as by

reason of deficiencies in facilities and infrastructure able to support investigations requiring reliable operation of highly sophisticated equipment.

One other potential player in development of biotechnology in service of agriculture in the developing countries is the class represented by indigenous private firms with interest in such application. In general, such firms are few or not present at all, but in countries like India or Argentina, entrepreneurial plant biotechnology-based companies (EPBCs) are appearing, to fill niche needs with techniques from cell biology, for example. Some of these companies eventually may look for connections with larger, more highly capitalized firms, as they find need for capital to expand their business, or need for broader research and development skills than they can afford to support.

International seed firms can interact beneficially with members of each category in the developing countries: national research programs, indigenous plant-biotechnology firms, and international agricultural research centers. This discussion initially will deal with ways ISCs and IARCs can interact in complementary fashion, to the benefit of developing nations.

Collaboration Between International Agricultural Research Centers and International Seed Companies

Collaboration between IARCs and ISCs can take place on three levels: research, technology transfer (i.e., commercialization), and joint policy development.

Biotechnology research needs in developing countries are large. Developing countries recognize the problem and are attempting to build up their capabilities, but as noted earlier they are limited in what they can do, individually. IARCs, therefore, could add greatly to the store of biotechnology knowledge useful to developing countries by establishing strong, well-rounded IARC programs in basic studies of cellular and molecular biology of their mandated crops, the same crops the developing countries would want to research.

To some extent, IARCs have already begun such programs. For example: CIMMYT has started work on restriction fragment length polymorphisms (RFLPs) for use in locating desirable quantitative trait loci in maize. CIAT has used meristem tip culture for development of an active *in vitro* germplasm bank of cassava. IRRI is looking at somatic variability in rice tissue cultures with hope of selecting salt- or cold-tolerant lines of rice.

But information available to me indicates that by and large the IARC biotechnology projects are applied rather than basic in nature and involve very little molecular genetics, being more oriented toward the kinds of cell and tissue culture work that can give immediately useful products. Much needs to be done in molecular genetics of the major food crops, whether for the developing or the developed world. Genetic transformation, transfer of functioning genes from one organism to another via steps involving DNA technology, will not yet work in any major food crop species except rice. Few agronomically important plant genes have yet been identified and cloned for use in transformation once the technology is workable in important food crops. Systems for gene regulation in the major food crops are largely unknown, molecularly. These problems urgently need solution; the bank of knowledge about the molecular biology of the major food crops in developing countries needs major additions of scientific capital.

The IARCs could be world-class centers of expertise and information exchange for the molecular biology of the developing world's major food crops and as such could help the developing countries to reach parity with the rest of the world in this field of basic plant science. They might develop such expertise in the science and become vital information transfer centers by collaborating scientifically with world-renowned biotechnology institutes in the developing countries such as the Max Planck Institute at Cologne (Federal Republic of Germany) or the USDA Plant Gene Expression Center at Albany, California. Having developed and shared such knowledge, IARCs then could work with applied plant breeding organizations (in addition to their own) to transform that basic knowledge into useful plant breeding products; they could be partners in the transfer of technology. The ISCs would be willing collaborators in such transfer.

(Of course, technology transfer from IARCs to the farmers of developing countries should be not only via ISCs. IARC technology should be transferred also through the national research programs and the indigenous seed and/or biotechnology companies. Each class of recipient: NARS, EPBC, and ISC will have its own technology needs in addition to the needs all have in common; consideration of the differing needs, the differing constraints to technology transfer, will stimulate research at the IARCs in ways that could benefit all recipients in the long run.)

One of the best ways to transfer biotechnological knowledge to practical plant breeding and variety development is to form teams of biotechnologists and plant breeders. For example, a team might be formed to use RFLP technology to identify valuable quantitative

trait loci in elite maize germplasm and then use that knowledge to breed better inbred lines of maize; or, a team could be formed to identify, with molecular means, important genes for disease resistance and then move them via genetic transformation technology into important crop varieties. Breeders and biotechnologists both would bring essential knowledge and skills to the work and would learn from each other as well, such that helpful suggestions could be made reciprocally and usefully.

IARCs and ISCs could collaborate to form such teams (as could also IARCs and NARS or IARCs and EPBCs) with useful commercial products, inbred lines and hybrids, the eventual result of such collaboration. But at this point a problem may develop—that of ownership of any resulting inbreds and hybrids, biotechnological processes, or molecular products such as cloned genes. It will be important for ISCs and IARCs to know what rights accrue to each collaborator in regard to products of joint research. For example, in the absence of assurance of exclusive licenses, ISCs could be reluctant to join in such collaborative research and technology transfer. This matter (intellectual property protection) is of equal importance to NARS and EBCs in the developing countries, as will be pointed out later in this essay.

Intellectual Property Protection and International Seed Companies

The nature of the private seed business requires that companies own or can license, uniquely, their stock in trade (their inbreds, hybrids and/or varieties) and, whenever possible, the processes, genes or plant parts that make their lines unique and hopefully superior to the competition. In the industrial countries with a highly developed private seed business, a body of laws and customs has evolved, and is still in active evolution, to allow investment in research and development of improved varieties to be repaid through sales of products protected with some combination of proprietary rights. Breeders' rights laws (European term) or plant variety protection laws (U.S. term) spell out terms of ownership for varieties and inbred lines; in the United States, recent rulings additionally have said that U.S. utility patent laws can be applied to plant varieties and inbreds, and also to hybrids, genes, plant parts, and processes connected with plant breeding.

But in most of the developing countries, breeders' rights protection is not available, and in fact its establishment often has been resisted as giving unfair and unwanted advantage to the transnationals, the international seed companies. Further, evaluation of infrastructure and social customs of many of the developing countries leaves

doubt as to whether effective administration of breeders' rights laws could be accomplished at the present time. Patent laws in some developing countries might be interpreted as allowing intellectual property rights over such things as processes or products of biotechnology applied to plant breeding, but to my knowledge, investigation of such potentials is hardly started.

Thus, a prerequisite for significant amounts of commercial collaboration between IARCs and ISCs (and, as well, with NARS and EPBCs) is development and implementation, country by country, of policy on intellectual property rights for plant germplasm and for processes connected with its manipulation. Further, in today's global economy, an international harmonization of such policy is a critical need. This will not be an easy matter, in fact, it will be an extremely difficult series of problems.

Even in the industrial countries, strong differences of opinion are developing, particularly over the question of ownership of breeding rights of released varieties and hybrids. Breeders' rights laws allow free use of released protected materials for breeding, continuing a custom as old as plant breeding. But the U.S. patent law has been interpreted by some to say that varieties, inbreds, and hybrids patented with the U.S. utility patent cannot be used (without the patent owner's permission) for breeding new commercial products during the life of the patent, although they can be used freely for experimentation, with the goal of learning how to make patentable improvements on the original patented product or process (the "experimental use" doctrine).

In general, seed companies without direct access to biotechnology as an aid to plant breeding advocate use of only one type of variety protection, the breeders' rights type, saying that this will guarantee free access to release protected materials for breeding. In contrast, seed companies with access to biotechnology (in-house or through their parent company) generally favor dual protection: breeders' rights and also the utility patent, saying that either or both methods might be used as appropriate to stimulate progress in plant breeding.

Members of the first group fear that without completely free access to released varieties and hybrids for breeding, the useful germplasm base would be dangerously restricted. Those of the second group say that genetic diversity actually would be increased in a beneficial way by wide-scale utilization of utility patents since each commercial organization would be stimulated to develop its own unique elite breeding populations (as is already done for development of proprietary inbred lines in the hybrid crops: maize,

sorghum, and sunflower). They say also that cross-licensing would allow a structured interchange of patented breeding materials as needed, so to some extent there would continue to be one large breeding pool per crop as is true today for crops such as soybeans or wheat. (However, the pool would have a broader base of elite materials due to greater diversity among the numerous unique private germplasm pools.)

It is clear that if there is as yet no agreement on ownership questions in the industrial countries with a history of experience in such matters, the matter is even farther from settlement in developing countries. But this is good reason for IARCs to concern themselves with the matter at this time. As parties with interests of the developing countries at heart, but also able to look objectively at the possible advantages of involving industry to the benefit of the developing countries, IARCs might provide a useful forum and some of the needed expertise for discussion of the matter, with a goal of helping to provide perspective and knowledge to those responsible for national decisions on application of intellectual property rights to plant germplasm.

Thus, research, commercialization, and policy development involving collaboration between IARCs and ISCs in matters of biotechnology and seeds requires (1) involvement of the IARCs in basic research in cellular and molecular biology, with possible joint research with ISC biotechnologists, and (2) formation of biotechnologist/breeder teams, composed of individuals from IARCs and ISCs, to effect technology transfer to the farm (commercialization). But, because any involvement with ISCs will need clear understanding about ownership of resulting commercially valuable products and processes, IARC/ISC collaboration also will require (3) efforts to assist developing countries, as asked, to devise workable policies for intellectual property protection of germplasm products and processes, policies with the best possible long-term benefit to the developing countries.

And, to repeat, commercialization of biotechnology-supported plant breeding to the benefit of developing countries requires that NARS and EPBCs also be involved, not only bilaterally with the IARCs, but also with the ISCs and with each other. In some instances, trilateral or quadrilateral relationships among the four groups will be useful. Such commercial interrelationships adjusted appropriately for each country, although seemingly complicated in concept and perhaps in execution, will in the long run give the most stability to agricultural development of the developing countries. And since commercial relationships require definition of property rights, it is obvious that matters of intellectual property protection applied to

plant varieties and hybrids, genes, processes, and plant parts are important to all sectors: IARCs, NARS, EPBCs, and ISCs.

Collaboration Between National Research Programs and International Seed Companies

ISCs also might collaborate with NARS in matters of biotechnology related to plant breeding. Some ISCs have considerable expertise in cell and molecular biology related to the products or crops they sell or plan to commercialize in some way. But also, during the past 10 years in the United States, numerous industry-university collaborations in biotechnology research have been crafted to allow the companies to extend their capabilities in biotechnology and its application to agriculture, and the universities to expand their funding base for research.

Typically, a company will fund a researcher or laboratory for a mutually agreed-upon area of research for a limited number of years. A pattern has emerged in which interests of both parties are protected—that of the universities for freedom in research and publication, and that of the companies for ability to commercialize resulting research products or processes, with protection provided, if needed, by patents.

In general, industry research grants to the universities stipulate that the universities will have first rights to apply for patents on products of that research, and the grantor, the company, has first rights to licenses on the patented items. License fees paid to the university are negotiated to the satisfaction of both parties. Publication is possible once patent applications have been made on discoveries deemed patentable.

Perhaps such arrangements, suitably modified, also could be made between ISCs and NARS. As noted earlier, patent protection for products and processes of biotechnology would need to be available; but, I believe that in some developing countries existing patent law may allow such protection. However, even if such protection is possible, its extension to varieties and hybrids containing patented genes or processes may not be permitted. So again, it is probable that broadening the scope of intellectual property protection applied to plants will be necessary before very much industry/national research collaboration in biotechnology can be instituted in the developing countries.

Industry also occasionally makes unrestricted research grants for quite basic types of plant research. Such grants typically are made to laboratories doing pioneering research that can advance the

entire scientific field. Since individual developing countries rarely are able financially to build up their laboratories to world leader class, they may find it difficult to attract grants of this category. However, they may be able to secure unrestricted training grants, occasionally supplied by industry for individual researchers, in order to improve their human resource capacity in advanced biological research.

Trade secrets are used by industry to protect technologies and/or other proprietary products. Sometimes, as partners or through a joint venture, two firms will share knowledge or jointly use trade secrets. Conceivably, such arrangements also could be made between ISCs and NARS. In Eastern Europe, ISCs and national or regional research institutes have made exclusive agreements of such a nature. For example, an institute can use an ISC's proprietary maize inbreds for production and sale of hybrid seed, with appropriate financial arrangements for both parties. Such agreements require a high degree of personal trust in addition to legal safeguards. They also require that the research institutes operate as *quasi* businesses, often in commercial competition with other institutes in the same country. Clearly, research centers in the national programs of developing countries would need to rethink their role and mission if they were to enter into similar partnerships or joint venture agreements with ISCs.

But, since in some cases nationally sponsored and/or subsidized research and development companies, fully commercial in intent, have been set up in developing countries, it may not take too great a stretch of imagination to see some of them in partnership with an ISC. The prime goal would need to be expeditious delivery of agricultural technology (in this case seeds or biotechnology, in support of seed breeding, production, and sales) to those in need of that technology. The developing nations would need safeguards to protect their economic and technological independence. The ISCs would need safeguards to ensure that their investments would not be unreasonably confiscated or rendered worthless by other means.

To review: collaboration between NARS and ISCs, more or less like that between IARCs and ISCs, could involve (1) collaboration at the research level (perhaps through research grants), (2) collaboration at the commercialization level (perhaps through partnerships or joint venture arrangements), and also would require (3) policy development in regard to intellectual property protection.

The Need for IARCs to Serve All Clients Impartially

One might, with a large stretch of imagination, also picture a trade secret-based IARC/ISC commercial partnership to effect technology transfer in seeds, including that from biotechnology. But this would seem to be very difficult to do without destroying the IARC's status as an impartial assistant or partner to the national programs of its client nations, and as an unbiased respondent with EPBCs and ISCs.

In general, the most uncomplicated and yet most productive way for IARCs to aid in technology transfer at the applied level is the method they already employ—to release elite breeding materials (produced with or without aid of biotechnology) to all qualified organizations and individuals. Such material can be at all levels of selection, from improved populations and F_2s on through several generations of selfing and selection. In those crops that can be grown as hybrids such as maize, sorghum, and sunflower, finished inbred lines could be released also, along with information as to their best heterotic combinations.

Once again, the question of ownership can arise, even with this practice of unrestricted release. In order to ensure that the general public has equal and unrestricted access to such materials, it would be well for IARCs to publicly disclose the origin and characteristics of each release in such a way as to preclude any unauthorized claims to them. An alternative is for IARCs themselves to obtain plant variety protection certificates or patents on the finished materials in countries where such is possible, following which unrestricted use of the protected lines is granted by the IARCs. However, this seems to me to be excessively burdensome; it generally cannot be done in those countries of most concern to IARCs and, at any rate, I doubt if it would be more effective than simple public disclosure, to ensure free and equal availability of materials.

Collaboration Between Indigenous Plant Biotechnology Companies and International Seed Companies

I mentioned earlier the possibility that indigenous entrepreneuring plant biotechnology companies sometimes might welcome arrangements with ISCs. In the United States, large agrichemical firms and, to a lesser extent, independent seed firms have made arrangements with entrepreneurial plant biotechnology companies. Types of association vary. They include research contracts, licensing agreements, equity investments, and joint ventures. Such arrangements might be feasible in developing countries, depending on local possibilities for foreign investment and control, and (as

usual) on local possibilities for intellectual property protection. Indigenous plant biotechnology firms could offer knowledge of the territory, local customs, and the intricacies of government bureaucracy, in addition to their specialized scientific knowledge and trained personnel. ISCs could contribute capital, a broader marketing potential, and a broader research base.

The Bases for Commercialization

Looking at all the possible commercial interactions among ISCs, IARCs, NARS and indigenous EPBCs, it seems to me one needs always to keep two salient points in mind: 1) developing countries need access to a solid base of basic plant science (including cellular and molecular biology) centered on their important food crops as grown in their local environments; and 2) developing countries need technology transfer from basic and applied plant science to the farm, via plant breeding, seed multiplication, and sales. This conference is exploring ways in which public and private institutions can collaborate to provide the science and expedite the technology transfer. I have listed some ways in which it might be done, with particular reference to the international seed companies.

Two Caveats, and Concluding Remarks

I think, however, that we must remember that influential individuals and organizations, networked in a worldwide coalition for social action, believe that for international seed companies, "transnationals," to have any part in the seed business of developing countries will be harmful to the people and the economies of those countries. Biotechnology in the hands of the ISCs is regarded as undesirable also.[1] And finally, any connection between IARCs and ISCs is looked upon with suspicion.[2] This is not the forum to review in detail the coalition's arguments nor to comment on them, but it is imperative that their criticisms and suggestions be kept

[1]"For almost a decade now, ICDA has been drawing attention to the dangerous narrowing of our food base and the impact of the increasingly monopolistic control of genetic resources in the hands of a few transnational corporations. . . . Biotechnology could be a powerful force for change in agricultural production. But it also could be the means by which monopolistic control over agriculture is increased." In *New Hope or False Promise? Biotechnology and Third World Agriculture*, by Henk Hobbelink, p. 6. The International Coalition for Development Action, April 1987.

[2]"...use of the IARC system might yet prove to be one of the few mechanisms that could reverse the privatization of biotechnology and challenge the direction of current research. . . . In the case of the Green Revolution, the TNCs acted "merely" as suppliers of the inputs for seeds that the IARCs had developed; for the biorevolution, the TNCs are direct competitors of the IARCs in bringing the technology to the farmers' fields." *Ibid.* p. 54.

clearly in mind during all considerations of technology transfer via commercial activities. The goal of The International Coalition for Development Action and allied individuals and organizations is the same as that of those assembled in this forum: to advance the agricultural capacity of developing nations to the benefit of all of their people. Differences of opinion as to how best to reach that goal should not be an excuse for fruitless disputation, but rather should be a basis for re-examination of proposed options, looking for previously unrecognized strengths or weaknesses, following which plans can be modified as needed and then put into action.

A second caveat also must be stated: biotechnology applied to plant breeding and other facets of agriculture will not bring about startling rapid advances in productivity on the farm nor windfall profits to agribusinesses. The superbly successful new sciences that are the basis for biotechnology will be invaluable aids to plant breeding and all successful plant breeders will use biotechnology as one of their important tools in the future, but its contributions will be evolutionary, not revolutionary, in nature.

The prediction is at variance with most of the public expectations for biotechnology, but many of its practitioners are in agreement with it. An important consequence of such knowledge is that planners in the developing countries should not place unwarranted reliance on biotechnology as a shortcut, as a way to bypass the slow, hard, and persistent work of standard plant breeding, seed multiplication, and distribution, integrated with improved agronomic practices. All of these processes still are needed to deliver the products of seed technology to the people of the developing nations.

But the planners' task, the task of those in charge of planning and implementing food strategies for developing nations, is to be sure that biotechnology is properly encouraged and smoothly integrated into plant breeding for the developing countries and that broadly-based systems of technology transfer, utilizing all appropriate institutions, are instituted promptly and efficaciously. International seed companies eventually may play a useful and significant role in this process as they interact, scientifically and commercially, with public and private institutions in the developing world.

National Programs in Biotechnology for Thailand and Other Southeast Asian Countries: Needs and Opportunities

Yongyuth Yuthavong
National Center for Genetic Engineering and Biotechnology
Ministry of Science, Technology and Energy
Department of Biochemistry, Faculty of Science
Mahidol University, Thailand
and
Sakarindr Bhumiratana
National Center for Genetic Engineering and Biotechnology
Ministry of Science, Technology and Energy
King Mongkut's Institute of Technology
Thonburi, Bangkok, Thailand

Introduction: Implications of Biotechnology for Development

Biotechnology makes many promises for the future. These promises carry deep implications, not only concerning economic benefits for those who own the technology, but also concerning the livelihood and development of people who will be affected by the new products and processes. For the developing world, biotechnology offers both threats and opportunities.[1,2,3] The threats are posed by, among other things, substitution of new products for the traditional ones which have been the sources of income for the developing countries. An example is the effect on the sugar industry, on which many developing countries depend for export income, of the new sweeteners produced by biotechnology.[4] The opportunities are offered by the fact that many developing countries are situated in the tropical belt rich in resources from which biotechnology could potentially provide value-added products. These opportunities can become realized once developing countries increase their capability in the technology.[5] Good policies and strategies for development and utilization of biotechnology can therefore have a very significant impact on the future of a developing country.

Opportunities and Needs

Thailand and other southeast Asian countries, constituting the Association of Southeast Asian Nations (ASEAN), are middle-stage developing countries with moderate to fast growth. The ASEAN region covers a land area of about three million sq. km., and has a total population of 309 million, with an annual growth rate of about 2%. The total GNP (1986) was U.S. $205 billion, and the average GNP per capita was about $675. The general

socioeconomic status of the ASEAN countries is given in Table 1.[6] For over two decades now, the ASEAN countries generally enjoyed a good socioeconomic development record and, although still relatively weak compared with the developed countries, are poised to become important members of the Pacific Rim in the year 2000 and beyond.

Table 1. Basic Socioeconomic Data of ASEAN Countries, 1986-7.

Country	Brunei	Indonesia	Malaysia	Philippines	Singapore	Thailand
AREA, 1000 sq. km.	5.8	1,919	329.3	300.4	2.6	514
POPULATION						
Size (million), 1987	0.2	174.9	16.1	61.5	0.6	53.6
% Average Annual Growth, 1979-86	2.6	2.1	2.4	2.8	1.1	2.1
Projected Year 2000	0.3	219.8	20.2	85.5	2.9	65.5
Death Rate per 1000	4	10	7	7	5	8
Birth Rate per 1000	30	31	31	35	17	29
Life Expectancy	62	58	67	65	71	63
ECONOMIC DATA						
Workforce (million)	0.1	63.8	5.6	20.9	1.2	27.9
GNP/GDP at market prices US$ b, 1986	3.42	86.47	26.34	30.13	17.97	40.18
Real GNP/GDP % Growth, 1986	-10	3.2	2.1	1.5	-1.8	3.4
Average real growth (1982-6)	-4.8	3.5	3.2	-1.1	7.1	4.1
Per Capita GNP (US$)	15,556	530	1,636	515	6,431	612

The ASEAN region is rich in natural resources, being the only region in the world which is both a net exporter of food (from Thailand) and of energy (from Brunei, Indonesia and Malaysia). Agriculture plays a very important role in the ASEAN economy, and will continue to be important despite recent trends towards industrialization. The advent of new biotechnology will likely enhance the role of agriculture, and help in its linkage with industries, especially those concerning food processing. This has substantial

implications, not only for ASEAN countries themselves, but also for industrially developed countries in joint venture and technology transfer for local production both for ASEAN and world markets.

Apart from the advantage of natural resources, the main opportunities in development of biotechnology in the ASEAN region are offered by a relatively low-cost skilled or semiskilled manpower base. A few examples from Thailand can illustrate this point. Plant tissue culture technology in Thailand has flourished commercially. Another example is the remarkable success of aquaculture technology. In Thailand, comparative advantage also exists for such downstream technologies as production of amino acids and antibiotics by fermentation processes. However, lack of highly qualified manpower still largely precludes the exploitation of such technologies as genetic engineering or hybridoma technology in the production sector at this stage, although many universities and research institutes are now using them for research purposes.

The needs for development of biotechnology in Thailand and other ASEAN countries arise from the general decline and fluctuations in commodity prices. These trends force the producers to lower the cost of production, to diversify and to increase the value-added portion of products, all of which require new approaches through biotechnology. For example, in order to reduce the cost of rubber production, there is a need to predict the potential yield of rubber at the young plant stage, through enzyme typing or other techniques, so that high-yield varieties can be chosen for planting which will require some five years to reach the production stage. Crop diversification can be achieved in the short term by focusing on import substitutes, for which technologies are well established. In Thailand, for example, the rapidly increasing demand for potato seeds can be met by plant tissue culture for production of mini- or microtubers. In the longer term, however, strategies for promotion of tropical fruits, medicinal herbs and other plants for which the ASEAN region is famous will bring a higher return, not only from the local, but also from export markets. The technologies for these plants are not well developed at present, and there is a need for Thailand and other ASEAN countries to collaborate with more developed countries in research efforts. The need for indigenous technology development is very well demonstrated here, because otherwise the result could well be crop diversification for the developed countries, with adverse effect on the developing ASEAN countries. In the longer term still, lesser-known plants of potential use in agriculture[7] can be explored, although there are many hurdles to be overcome in introducing new crops to the market. Cassava starch provides a good example whereby various value-added technologies, mostly well established in the developed

countries, such as enzyme technology, carbohydrate modification technology, and fermentation technology, can be utilized following successful technology transfer. In other areas, joint research and development are needed, as for conversion of palm oil to more valuable products through transesterification and other procedures.

Status and Impact of Biotechnology in Thailand

The importance of biotechnology to Thailand has been realized for over a decade now. The status and impact of these technologies has been the subject of several analyses.[8,9,10] Although the infrastructure for biotechnology in Thailand is relatively strong in comparison with other developing countries, it still has a very small number of qualified scientists in this specific area. Thailand has some 60,000 scientists and engineers at degree level, or 11 per 10,000 population. However, it can be estimated that there are fewer than 500 with at least a master's degrees who are active in biotechnology research, and most of these are in the universities. Current degree programs produce some 40 bachelor's degree graduates per year in biotechnology, and another 60 per year in food science and technology. The combined production capacity for agriculture, medicine and other programs related to biotechnology is about 1,700 bachelor's per year. The production capacity for master's degrees in biotechnology is about 30 per year, and the combined production capacity for allied sciences is about 330 per year. The doctoral degree programs in life sciences presently exist in three universities, with the highest strength in Mahidol University which has up to now produced some 50 graduates during the past 20 years. There is virtually no post-doctoral system in Thailand at present.

Research and development in biotechnology, as in other areas, are mostly done in the universities. Mahidol University has various active research groups, with emphasis on molecular biology and genetic engineering of various biomedical areas, especially those concerning tropical diseases and industrial microbiology. Kasetsart University is active in various areas of agricultural biotechnology, including tissue culture for improvement and propagation of various plants, embryo transfer technology, and fermentation technology. Chulalongkorn University is well known for research on aquaculture of shrimps, rice tissue culture, and processing technologies. King Mongkut Institute of Technology in Thonburi has various activities on bioengineering and biomass utilization. All of these institutes are located in the Bangkok area. Among the provincial universities, Chiang Mai University is the most active, with works on tissue culture of various crops, including orchids, teak and potato, and postharvest biotechnology.

Among the government agencies, the Department of Agriculture has a very large research program, much of which is related to biotechnology. An A.I.D.supported project enabled the production of Rhizobium on a large scale. Specialized research institutes in this department deal with rice, rubber, and other crops. Other departments which have research with biotechnology components include the Departments of Livestock, Fishery and Forestry. The Thailand Institute for Scientific and Technological Research, a government-funded institute operating as a public enterprise, also has many activities in the biotechnology areas, mostly concerning industrial microbiology.

The private sector in Thailand, to which agriculture and agro-industries are very important, already uses biotechnology substantially for their products and processes. Examples of various biotechnologies are given below.[11]

A. Agriculture

Ornamental flower production: A number of small companies produce orchids and other ornamentals through micropropagation in tissue culture worth about $18.5 million/year.

Rhizobium production: The Department of Agriculture produces 70 tons/year for distribution to farmers.

Embryo transfer: Cattle industries have started to use the technology. Estimated saving of imported cattle: about $2 million/year.

B. Industry

Food components production: A lysine production plant established; estimated market: about $2 million/year. Local production of citric acid through an improved method on trial; estimated market: about $4 million/year.

Glucoamylase production: Local production on experimental trial; estimated market: about $4 million/year.

C. Energy and environment

Waste treatment: Treatment of waste from cassava starch factories.

D. Health

Diagnostics: Many small companies are engaged in local production. Total market estimate: about $20 million/year.

Antibiotics: First factory in southeast Asia produces kanamycin (10 tons/year) from agricultural raw materials.

Problems and Constraints in the Development and Application of Biotechnology

A. *Manpower.* Although there is an infrastructure for qualified manpower production in most ASEAN countries, this is presently not adequate as the data above show. The inadequacy is not only in quantity, but also in quality since biotechnology is a very fast-moving field. A recent survey showed that the content of biotechnology programs offered in the universities in Thailand severely lacks new aspects of the technology which are responsible for its present prominence.

B. *Technology development capability.* With insufficient manpower and lack of access to new technology, the indigenous capability for technology development is limited. Although the problems for ASEAN countries, with open economies and easy communication with developing countries, are not as severe as for many other developing countries, they are substantial and need specific policy measures to overcome. One main problem is the lack of research and development activities in the private production sector, and another is the lack of linkage between the private sector and the university and government sectors which are the major performers of research and development.

C. *Intellectual property rights.* Patent laws in ASEAN countries reflect the status of industrial development, and have to be improved as the development becomes more advanced. Protection of biotechnology inventions require various unique considerations chiefly due to the fact that they deal with living organisms, and have only recently started to be clarified in the developed countries. These issues will gain increasing importance as biotechnology becomes more important in ASEAN industries. Although patent systems aim to further early disclosure and dissemination of knowledge embodied in the invention, there is a reluctance to strengthen the system rapidly, due to the fear that this will hamper indigenous technological and industrial development. This fear is reinforced by the fact that a great majority of the patents are granted to foreign inventors. For biotechnology, an added complication is the fear that the local genetic resources might, after some modification by inventors in developed countries, be protected from use by the local industries themselves.

D. *Use and conservation of bioresources.* A dilemma is faced by the bioresource-rich ASEAN countries in collaboration with the

developed countries. Put simply, the main problem is that of balancing between the benefits of technology transfer from, and the cost of transfer of genetic resources to, the developed countries. On top of this are the problems arising from the impact of the use of new biotechnology. For example, what effects would the use of herbicide-resistant crops have on the ecosystem? What are the effects on the total gene pool of excessive use of newly developed plant or animal varieties?

E. *Biosafety*. The regulations and regulation enforcement issues concerning biotechnology have hardly been touched in the ASEAN countries. Due to strict and complicated regulations in the U.S.A. and other developed countries, technology developers in these countries might increasingly look to ASEAN countries as venues for field trial and environmental release of new products. Furthermore, many indigenous research and development activities require new guidelines concerning safety to the public and the environment. There is a need therefore to strengthen the regulatory system in ASEAN countries in anticipation of future development.

National Policies and Programs

Thailand and a few other Southeast Asian countries have specific policies and measures to promote the development and application of biotechnology. Singapore has various schemes to help finance research and development activities and commercialization in the private sector, including the Research and Development Assistance Scheme, the Product Development Assistance Scheme, and various tax incentives. The Science Park was recently established near the National University of Singapore, and the Institute of Cell and Molecular Biology has been set up to do research at the leading edge. The Philippines, Malaysia, and Indonesia also have biotechnology plans and programs at the national level. The six ASEAN countries have recently formulated regional programs under the auspices of the Committee on Science and Technology.

Biotechnology development programs started in Thailand in the late seventies with the formation of centers in important universities. In 1983, the government set up the National Center for Genetic Engineering and Biotechnology to be the main policy, support and coordination center for biotechnology research and development projects, and to form linkages with the private sector. The establishment of the National Center marked, for the first time, significant local funding from the government towards development of a specific technology. Support for the technology is, moreover, provided as a complete package with provision of funding, information, training, linkage to industry, and international affiliation. The

National Center has four affiliated laboratories, including pilot plants, and over 30 projects in nine institutions in the network. The emphasis is on development, transfer and utilization of biotechnology, including genetic engineering in the following areas: industrial, agricultural, public health, energy and environmental applications, and strengthening of basic infrastructure required for genetic engineering and biotechnology. Under these guidelines, the National Center commissions various studies on the status of specific technologies or industries to assess the economic and social importance of the specific areas and to pinpoint the research, development and technology transfer needs to be fulfilled. Designated research projects are then formulated by researchers, often from more than one institution working together, and are funded after proper peer review. Table 2 gives a summary description of the various projects supported by the National Center, and Table 3 gives a summary of works undertaken at the four affiliated laboratories (see end of text).

The Science and Technology for Development Program,[12] established in 1985 with the cooperation of the U.S. Agency for International Development (USAID), is another major supporter of biotechnology research and development in Thailand. The Science and Technology Development Board (STDB), which runs this program, has a wide interest covering bioscience and biotechnology, material technology and applied electronics and computer technology, areas which were determined to be of high priority by previous studies.[13] Other biotechnology research projects are supported by the National Research Council and by grants from the universities. International funding is also significant, especially for biomedical and life sciences projects.

International Collaboration in Research, Development and Commercialization of Biotechnology

Thailand and other ASEAN member countries highly value international collaboration in research, development and commercialization in biotechnology. The ASEAN countries have various collaborative projects with Australia in biotechnology related to food and food waste utilization. Among bilateral programs, the STDB program places emphasis on interaction with counterparts from the U.S.A., both scientists and commercial partners. U.S. scientists also played a significant part in the early development of ideas for collaboration with the National Center for Genetic Engineering and Biotechnology in Thailand.[14] USAID has also helped the National Center in the studies toward formation of the national network for plant tissue culture technology. In addition, the National Center also has close links with the U.K., Australia, Japan, the Netherlands and other countries.

Collaborative programs between the National Center in Thailand and agencies in the U.K. and Australia will be given as examples. In the Thai-U.K. program, emphasis is placed on the development of enzyme technology, with exchange of scientists, provision of fellowships to Thai students to do a part of their research in the U.K. while pursuing Ph.D. degree in Thai universities, and collaborative research projects. The Thai-Australia program puts emphasis on joint commercial exploitation, with respective central agencies acting as matchmakers in joining commercial partners from the two countries.

There are vast opportunities for further international collaboration. The U.S.A., as the world leader of new biotechnology, should be a very important partner in the collaboration. For Thailand, two major directions in the collaboration with the U.S.A. should be in the acquisition of new technologies, especially in molecular biology and process engineering, and in joint commercial applications using complementary advantages of the two countries. STDB and the National Center can act as coordinating and supporting agencies for collaborative programs. In addition to supporting collaborative research projects, STDB also has an industrial development support program with provision of special loans to industries for ventures in new technologies. The private sector also has access to new venture capital from new international and local sources.

Conclusion

National policies and programs on biotechnology in Thailand demonstrate the current awareness and optimism in the Southeast Asian countries regarding benefits of new technologies. While these policies and programs still need to be developed further, they provide ample basis for international cooperation in various forms. Agriculture is the most important component of our national programs, reflecting its importance in the economy. The private sector has a large role to play in the development of biotechnology as a whole, and of agricultural biotechnology in particular. A few problems remain to be overcome regarding technology transfer, sharing of benefits from intellectual and genetic resources, and regulations concerning biosafety. These problems are being tackled in Thailand through the National Center for Genetic Engineering and Biotechnology, the Science and Technology Development Board, and other relevant agencies. The needs for and opportunities in development of biotechnology in Thailand are so substantial that the problems seem small by comparison.

Acknowledgements

We thank the USAID and the Science and Technology Development Board, Thailand, for support in attending this conference.

Table 2. Summary Description of Research Projects Supported by the National Center for Genetic Engineering and Biotechnology.

Projects	Description
I. Industrial Applications	
1.1 Research and Development for the Industrial Production of Glucoamylase and α-Amylase. (Mahidol University)[a]	The major objective is to develop local capability in industrial production of glucoamylase and α-amylase. Two fermentation procedures were carried out for the glucoamylase production. They are the pilot scale solid substrate fermentation utilizing *A niger* MUCA-8 and the laboratory scale submerge culture fermentation utilizing *A niger* KUB-1. Two strains of *Bacillus sp.* KUB-B[1] and KUB-B[2] are being investigated at laboratory scale level for the production of α-amylase.
1.2 Production of Pure Glucose for Pharmaceutical Purposes. (Chulalongkorn University)	The objective is to develop technology for the production of injectable grade glucose which is currently imported at a very high value annually. The production of injectable grade anhydrous glucose with universal standard specifications was achieved at a bench scale. Equipment for pilot scale production were designed, fabricated and tested. Pilot scale production is being carried out to obtain data for industrial scale production.
1.3 Production of 6-Aminopenicillanic Acid by Biotechnological Processes. (Mahidol University)	The activities include strain improvement for increased enzyme production, optimization of the enzyme production process, searching for appropriate methods for enzyme and 6 APA purification, immobilization of enzyme and cells and bioreactor design. Cloned PAC genes of *B. megaterium* UN1 and *E. coli* 194-3 were studied which included the restriction mapping

[a]Parentheses indicate the principal institution carrying out the research.

Table 2. Summary Description of Research Projects Supported by the National Center for Genetic Engineering and Biotechnology.—Continued

Projects	Description
	and subcloning of both genes. Attempts to join strong promoter to the PAC genes to increase the enzyme production are being made. Immobilization of E. coli is studied using alginate, cellulose acetate, and cotton cloth. A low-cost medium for enzyme production has been formulated.
1.4 Citric Acid Production in Submerged Culture. (Kasetsart University)	Thailand's local citric acid production using solid substrate fermentation satisfied about 50% of total local demand. This project aims at developing a technology of efficient citric acid production method using submerged culture and a technique for purification of citric acid for medical usage. The target is to obtain a commercially viable concentration of 9-12%. The system developed within this project has produced 13-15% citric acid from cassava starch in a laboratory scale fermenter. Scaleup study is underway for a stirred tank and an airlift fermenter.
1.5 Development of Wine Production from Wine Grapes. (Kasetsart University)	This recently developed project aims to study the wine production process and to test the suitability of wine grapes grown in Thailand for wine production.
1.6 Improvement of Yeast Strains for Production of Food Yeast and Yeast Autolysate. (Chiang Mai University)	The project aims to select and improve yeast strains for the production of yeast and yeast autolysate utilizing cassava starch as raw material. Standard strain selection methods was carried out. Attempts will be made to transform amylase gene into C. *utilis*.
1.7 Production and Utilization of High Test Molasses in the Fermentation Industry. (Thailand Institute of Scientific and Technological Research)	High test molasses is superior to black strap molasses as raw material for fermentation and can be produced in current sugar mills with only minor adjustment. Technology to produce high test molasses from sugarcane was developed. It was also shown by the project that it is feasible to replace black strap molasses by high test molasses as raw material for ethanol production. Market study on the production of high test molasses by the existing sugar miller was carried out.

Table 2. Summary Description of Research Projects Supported by the National Center for Genetic Engineering and Biotechnology.—Continued

Projects	Description
1.8 Design of a Pilot Plant to Produce SCP from Cassava. (King's Mongkut Institute of Technology, Thonburi, and Thailand Institute of Scientific and Technological Research, TISTR)	A pilot plant to produce feed grade SCP from cassava was designed. The pilot plant was designed as an attachment to the existing 1,500 litre/day alcohol pilot plant of TISTR. The design was based on results of a 370-litre airlift fermenter using *S. cerevisiae 281*. Preliminary economic study showed that the major cost of production was the price of cassava roots.
1.9 Antibiotic Production for Use in Animal Feed. (Kasetsart University)	The major aims of the project are to select microorganisms which can produce antibiotic substances which are effective against a wide range of diseases and to develop technology for the production of the antibiotic. Two isolates have been obtained and are currently being investigated.
II. *Agriculture Applications*	
2.1 The Development of Embryo Transfer Technology in Dairy Cattle and Its Application under Thailand Condition. (Kasetsart University)	Embryo transfer technology has been developed in Thailand with two main objectives: to increase the rate of milk production and to improve dairy cattle breed by developing genetically superior livestock that will yield maximum milk and meat production under Thailand's condition. Many calves were produced from frozen imported embryos using nonsurgical transfer techniques into native recipients. Other calves were born from fresh embryos produced under Thailand's condition. The adaptabilities and performances of offsprings born from frozen embryos of exotic breeds will be determined.
2.2 Steroid Immunization to Recover Fertility in Swamp Buffaloes at Village Level. (Chulalongkorn University)	Since swamp buffalo commonly shows irregular cycling and subnormal fertility, a method for improving ovarian activity and fecundity is needed. Hormone Immuno Neutralization (HIN), using steroid immunization, was investigated as a possible means of stimulating ovarian function in the swamp buffaloes. A strategy and a system of HIN in swamp buffaloes at small farm level was established and is being assessed by the percentage of increased fertility. A total of 3,758 females were studied.

Table 2. Summary Description of Research Projects Supported by the National Center for Genetic Engineering and Biotechnology.—Continued

Projects	Description
	It appears that HIN will become perhaps the most important tool to efficiently regulate the endocrine system of the female and will provide a basis for more successful artificial insemination and embryo transfer.
2.3 Strain Selection and Cultivation of Terrestrial Snails. (Mahidol University)	In Thailand, terrestrial snails are plentiful and of high economic value as export items. Among the many species of land snails, *Achatina fulica* has been the most popular export item. However, the dark flesh of the species has not been popular among the Thais. The aim of the project is to systematically cultivate the snails in order to select strains of albino snails through crossbreeding and to increase the growth and reproduction rate of the snails by identification, isolation, and priming of the hormonal factors. These factors and the study of the snails' reproductive physiology are being conducted.
2.4 Development of Technology for Commercial Production of Disease-free Potato Seeds. (Kasetsart University)	The project's major aim is to develop technology for commercial production of disease-free potato seed in an attempt to replace the nearly 200 tons current annual import of the seeds. Virus-free potato plant material was successfully produced by the apical meristem techniques. Disease-free mini- and microtubers were also successfully produced. The tubers are potentially suitable for large commercial scale production. Field trials are being carried out.
2.5 Technological Development of Industrial Horticulture Crop Production. (Kasetsart University)	The major aims of the project are to apply tissue culture technology for the development of temperate cut flowers production and to produce disease-free ginger seed for growers. Mass propagation by tissue culture technique was carried out for several varieties of temperate cut flowers, e.g., chrysanthemum and lily. Gerbera hybrids between Thai and European strains were produced and the selected plants were mass propagated using tissue culture technique. New gamma-induced varieties of chrysanthemum were introduced.

Table 2. Summary Description of Research Projects Supported by the National Center for Genetic Engineering and Biotechnology.—Continued

Projects	Description
2.6 Conservation Technology of Unique Characteristic Plants *in vitro*. (Kasetsart University)	The major aim is to develop technology for conserving plants of importance to Thai culture. Tissue culture technique was applied to several selected plants which included *Artocarpus heterophyllus* (jackfruit) and *Ervatania coronaris*.
2.7 *In vitro* Propagation and Screening of *Indigenous Costus* lacerus for High Diosgenin Production Cultivars. (Mahidol University)	The major aim is to select varieties for high production of diosgenin. Chemically screened *Costus lacerus* were mass propagated by *in vitro* techniques. Multishoot was obtained by culturing in a nodal segment in Murashige and Skoog's medium supplemented with benzyladenine. Vigorous plantlets obtained were transplanted. Rates of diosgenin synthesis and accumulation thereof are being compared between the mature rhizomes of tissue culture-derived and conventionally propagated plants.
2.8 Plant Tissue Culture Biotechnology Network. (The National Center)	The network was set up for coordinating the research and development among many institutes active in the field of tissue culture. Several technical workshops were organized cooperatively. Personnel and Plant Tissue Culture Data Bases have been set up. "Plant Biotechnology" newsletter has been published quarterly.
2.9 Research and Development on Rattan Production. (Kasetsart University)	The major aim of this project is to study factors affecting the growth and development of rattan in an attempt to speed up the propagation of rattan. Tissue culture technology has been applied and systematic study of rattan pest and disease are studied.
2.10 Application of Tissue Culture Technology for the Improvement and Propagation of Oil Palm. (Prince of Songkla University)	The unavailability of good quality oil palm seedling has inhibited the development of the Thai palm oil industry. The project aims to apply tissue culture technology to mass propagate selected oil palm and subsequently to improve upon the quality of the palm.

Table 2. Summary Description of Research Projects Supported by the National Center for Genetic Engineering and Biotechnology.—Continued

Projects	Description
2.11 Association between Rice (*Oryza sativa* L.) on Nitrogen Fixing Bacteria. (Chulalongkorn University)	An ability to reduce fertilizer requirement on rice production can have a major impact on the Thai economy and the earning ability of rural farmers. The project aims to study the association between rice and the nitrogen fixation bacteria. Chemotaxonomy of the associative bacteria attaching to the root surface and contained within the rhizosphere were studied. Initial results have indicated that rice lectin may play a role as an associative factor.
2.12 Research and Development on Microorganisms for Compost Production in Thailand. (Kasetsart University)	More than one hundred strains of cellulolytic microorganisms were isolated. Their effectiveness in the production of compost from plant residue under field conditions are being investigated in comparison with commercially available strains. Searching for an appropriate technique for testing microorganisms to assist the consumer in evaluating the effectiveness of the commercially available strains is one of the aims of this project.
2.13 Research and Development on the Utilization of Vesicular-arbuscular Mycorrhiza and Their Combined Effects with Nitrogen-fixing Bacteria in Legumes. (Thailand Institute of Scientific and Technological Research)	Vesicular-arbuscular (VA) mycorrhiza fungus is known to thrive well in symbiotic association with the plant roots and to increase plant productivity by improving phosphate uptake (reducing the fertilizer requirement). Several strains of VA-mycorrhiza have been isolated from legume planting areas. Preliminary assessment on the efficiency and the development of strains associated with the plant (*Sorghum bicolor*) has indicated the strains from *Glomus spp.* to be superior to other strains tested.
2.14 Screening and Improving Genetic Potential of N_2-Fixing Blue Green Algal Strains for Use as Biofertilizer to Improve Rice Yield. (Thailand Institute of Scientific and Technological Research)	The developmental objective of this project is to improve the productivity of rice production and to reduce the cost associated with fertilizer requirement. Nearly 1,000 paddy soil samples from all over Thailand were collected and the occurrence of N_2-fixing blue-green algae were investigated. Eighty two strains of the algae were isolated and tested for growth and N_2-fixing ability. Among other factors studied were the effect of salt and pesticide (Propanil)

Table 2. Summary Description of Research Projects Supported by the National Center for Genetic Engineering and Biotechnology.—Continued

Projects	Description
	on the growing of the algae. Field experiments on the effects of the biofertilizer on rice yield will also be carried out.
2.15 Biotechnology Technique for Selection and Breeding of High-Yielding Rubber Clones. (Prince of Songkla University)	The main objective is to develop a reliable technique for yield evaluation of rubber trees at the seedling stage. A good-yielding tree will result in substantial additional output over the average tapping period of more than 30 years. It was found that the activity of the hydroxy-methyl-glutaryl Coenzyme A reductase in latex tapped from mature trees correlates well with rubber yield. The same correlation was found in leaves detached from two different clones of rubber seedlings, whereas the activity level of mevalonic acid kinase showed no correlation with rubber yield.
2.16 Development of Biotechnology for Cultivation of Shiitake Mushroom (*Lentinus edodes*). (Chulalongkorn University)	Thailand annually imports a large amount of shiitake mushrooms. Until recently the mushroom could only be cultivated on the highland in Northern Thailand at the expense of the precious oak tree. The project aims to develop the cultivation of the mushroom using agricultural compost. Substrates for the cultivation were successfully developed from para-rubber tree sawdust, rice bran and essential supplement extracted from oak sawdust. Factors influencing bag production of the mushroom were studied. Several strains with desirable characteristics were isolated and physiological studies were conducted.
III. *Public Health, Energy and and Environmental Application.*	
3.1 Field Trials of Mosquito Control Using Spore-forming Bacteria in Thailand. (Mahidol University)	Field trials of mosquito control using spore-forming bacteria have been carried out in an area in Bangkok as well as in rural areas where cases of malaria were found. The study has indicated that the *B. sphaericus 1593* gave a good control effect on *A. minimus* larvae for approximately three months while the *B. thuringiensis H-14* was effective against *A. albopictus*. Cur-

Table 2. Summary Description of Research Projects Supported by the National Center for Genetic Engineering and Biotechnology.—Continued

Projects	Description
	rent research is concentrated on the minimizing of the cost of production of the microorganism and searching for effective means for dispensing the microorganisms.
3.2 Conversion of Solid Waste to Methane by Two-stage Anaerobic Digestion. (Chulalongkorn University)	Solid waste from fresh market were digested to produce biogas utilizing a two-stage anaerobic digestion technology. Effects of operating parameters were studied.
3.3 Utilization and Treatment of Tapioca Starch Factory Effluent. (King Mongkut Institute of Technology, Thonburi)	The major aim of the project was to produce valuable products from tapioca starch factory effluent. Bench scale experiments have led to two pilot production systems for the production of biogas for use as fuel in the starch factory and the production of Spirulina for animal feed. Estimated internal rate of return for a full-scale biogas plant was shorter than five years in most cases. Commercial scale production of spirulina would depend on marketability of the product.
3.4 Preparation of *Opisthorchis viverrini* Antigens by Genetic Engineering Techniques for Use in Immunodiagnosis. (Mahidol University)	The project aims to produce the specific antigen for *O. viverrini* by rDNA Technology using *E. coli* as host organism. It was found at this early stage of the project that it was possible to prepare RNA and construct cDNA without requirement for the poly (A) tail of mRNA.
IV. *Strengthening of Basic Infrastructure*	
4.1 The Preparation of Genetic Engineering Materials as a Service. (Mahidol University)	This project aims to stimulate the development of genetic engineering in Thailand through the preparation and distribution of some basic genetic engineering materials as a service. The following materials have been prepared: restriction endonucleases (EcoRI, PstI and BamHI), plasmid vectors (pBR 322, pBR 325 and pUN 121), DNA marker (pCB1-6), and T_4 DNA ligase.

Table 2. Summary Description of Research Projects Supported by the National Center for Genetic Engineering and Biotechnology.—Continued

Projects	Description
4.2 Research and Development of Cell Technology for Production, Collection, and Dissemination of Mammalian Cell and Tissue Cultures. (Mahidol University)	The major objectives are to develop a mammalian cell culture depository to serve the increased demand in Thailand and to provide training in tissue and cell culture handling and manipulation. Thirty-four cell lines are currently available. Techniques for primary culture of a number of mammalian tissues were developed.
4.3 Microbial Culture Collection and Services Program. (Thailand Institute of Scientific and Technological Research)	The main purposes of the program are to collect and preserve microorganisms, to supply cultures on demand, and to help and assist researchers seeking strains which are not available in Thailand. The methods of preservation use are lyophilization and storage in liquid nitrogen.
4.4 *In vitro* Germplasm Collection and Exchange of Economic Plants. (Kasetsart University)	The program aims to carry out research and development on tissue culture technology as applied to the development of plant seeds and to use cryopreservation technology in preserving germplasm as well as act as a center for exchanging of germplasm. Germplasm of species of sugarcane, papaya, soybean, tomato, fruit trees, and other cash crops are to be available by 1990.
4.5 Development on the Production of Glucose and Fructose from Sucrose by Chromatographic Methods. (Kasetsart University)	Project 4.5 -4.7 are small projects aimed at fostering the development of manpower resources in biotechnology. The project may later develop into a larger program under the three areas of application.
4.6 Study of Methods for Agarose Production from *Gracilaria spp.* in Thailand. (Srinakarinwirote University)	
4.7 Technological Development for the Commercial Production of Viral Insecticide. (Kasetsart University)	

Table 3. Summary of Works Undertaken at the Affiliated Laboratories.

Affiliated Laboratories	Activities
1. Plant Genetic Engineering Unit at Kasetsart University	Organized in 1985, the unit is active in the area of development of plants of economic importance to Thailand resistant to diseases and adverse conditions, and the development of pest protection techniques. Current activities include development of papaya resistance to the ring spot virus and tomato resistance to the yellow leaf curl virus. In addition, the unit regularly organizes seminars and workshops on various aspects of plant biotechnology.
2. Microbial Genetic Engineering Laboratory at Mahidol University	Organized in 1985, the unit is active in many areas of genetic engineering. Current activities include the success in cloning and expression of mosquitolarvicidal δ-endotoxin gene of *B. thuringiensis* in *E. coli* and the development of DNA probes for the detection of microorganisms which cause fish disease. The Unit has also produced highly specific and sensitive DNA probes for detection and classification of mosquitoes and malarial parasites. National seminars on genetic engineering and DNA probe technology are organized regularly.
3. Marine Biotechnology Laboratory at Chulalongkorn University	Organized in 1986, the laboratory is active in the development of aquaculture technology and the utilization of marine resources. The current research topics include improvement of technology for oyster culture, improvement of seabass production using bacterial vaccine, strain improvement of giant prawn by genetic manipulation, and physiological study of selected strains of *Spirulina spp.*
4. Biochemical Engineering and Pilot Plant R & D Unit at King Mongkut's Institute of Technology, Thonburi	Organized in 1986, the unit is active in research and development in engineering and processing fields as related to biotechnology. Pilot equipment is designed, fabricated, and used as research and training tools. Bioreactors, separation techniques, and process instrumentation and control are among the areas of specialization. Specific examples are the development of an Industrial Biogas Project utilizing agroindustrial waste, development of pilot scale airlift fermenter and membrane reactor, process modelling and control of fermentation process. Pilot plant training courses have been regularly organized.

References

1. UNESCO (1987), *The Courier*, March 1987.

2. Ahmed, I. (1988) The bio-revolution in agriculture: Key to poverty alleviation in the Third World? *Int. Lab. Rev. 127*, 53-72.

3. Dembo, D. and Morehouse, W. (1987) Trends in biotechnology Development and Transfer. 1 PCT 32. UNIDO, Vienna, pp. 96.

4. van den Doel, K. and Junne, G. (1986) Product substitution through biotechnology: Impact on the Third World. *Trends Biotechnol.*, April, 88-90.

5. McConnell, D., Riazuddin, S. and Zilinskas, R.A. (1986) Capability building in biotechnology and genetic engineering in developing countries. IS 608. UNIDO, Vienna, pp. 114.

6. Far Eastern Economic Review (1988). *Asia Year Book 1988*.

7. Vietmeyer, N.D. (1986) Lesser know plants of potential use in agriculture and forestry. *Science 232*, 1379-1384.

8. Yuthavong, Y., Bhumiratana, A. and Suwana-adth, M. (1984) The status and future of biotechnology in Thailand. In *Proceedings of ASEAN-EEC Seminar on Biotechnology: The Challenges Ahead*. Singapore, November 1983, Science Council of Singapore, pp. 29-39.

9. Yuthavong, Y. (1987) The impact of biotechnology and genetic engineering on development in Thailand. *J. Sci. Soc. Thailand 13, 1-8*.

10. Suwana-adth, M. (1987) Thailand's position in relation to biotechnology and bioindustrial development. Proceedings of the Conference "First ASEAN Science and Technology Week", April 1986, Kuala Lumpur, Vol. II, pp. 239-246.

11. Yuthavong, *ibid.*

12. USAID (1985) Science and Technology for Development. Project paper (493-0340) USAID/Thailand, Bangkok.

13. Yuthavong, Y., Sripaipan, C., Kirtikara, K., Glankwandee, A. and Trakulku, K. (1985) Key problems in science and technology in Thailand. *Science* 227, 1007-1011.

14. BOSTID (1986) Development of a national center for genetic engineering and biotechnology in Thailand. National Academy Press.

Biotechnology: New Approach for Agricultural Research, Training and Development at CATIE

Victor M. Villalobos
CATIE
Costa Rica

The Tropical Agriculture Research and Training Center (CATIE), began its activities at its present site at Turrialba, Costa Rica, in 1942 under the name of the Interamerican Institute for Agricultural Sciences (IICA). In 1973, following the transference of the IICA headquarters to San Jose, the Center was reorganized as a regional training and research center for member countries of the Central American isthmus and the Dominican Republic. Presently, the center has three basic functions: research, teaching, and agricultural development organized to support the small farmer.

In order to face the agricultural reality of the region; CATIE recently modified its structure to embrace three general programs: 1. Improvement of Tropical Crops; 2. Production and Sustained Agricultural Development; and 3. Integrated Management of Natural Resources. The interdisciplinary approach of these three programs proposes new strategies for the solution of agricultural problems of the region through continuous involvement with national programs and through the establishment of pilot research areas in the member countries.

Tropical Crops Improvement Program

This program addresses the improvement of three perennial crops of major importance in the region and considers future diversification using promising new tropical species. The three perennial crops are coffee, cocoa, and plantain (cooking banana). Existing collections of valuable plant genetic resources are also being used to evaluate other promising species.

CATIE has gained a reputation over the years for its work on coffee and cocoa, two major export crops for the region. Recently, plantain has been added as another priority crop. At present no other institution in the region plays such an important role in the improvement and conservation of these species.

The program direction and focus are strongly related to the improvement of coffee, cocoa, and plantain, particularly by obtaining highly productive and disease-resistant genotypes. The collection, maintenance, evaluation, and distribution of promising tropical

plant genetic resources also will be intensified through technology development.

Using a combination of conventional improvement methods and biotechnology, CATIE should be able to accomplish these objectives in a shorter period of time, during which technology development and experimentation will be an important activity. This program represents an area of excellence for CATIE and a unique opportunity for major impact on agricultural production in the region.

The Role of CATIE in Using Biotechnology in Tropical Research

The response to a survey carried out in 1986 on the actual state and perspectives for biotechnology research in Latin America and the Caribbean (Roca *et al.*, 1987), made it clear that great hopes and importance were being given to the contribution that applied biotechnology could make to the region's agriculture. The survey confirmed the existence of national programs to deal with problems in the reproduction of desirable genotypes, the elimination of viruses in vegetatively reproduced species, and genetic improvement and conservation of germplasm. Nevertheless, this survey and other studies carried out in the region showed that tropical species of great importance were not receiving the attention being given to many temperate species.

Research carried out in countries outside the region has revealed considerable evidence of the comparative advantages of using biotechnology in tropical crops (Litz *et al.*, 1984; Gupta *et al.*, 1986; Yeh and Chang, 1986, etc.). In support of these advantages, three important aspects must be recognized: first, the species that grow in the tropics are of great economic importance in the region and have considerable export potential; second, researchers in the temperate zone who have advanced techniques at their disposal are rarely interested in unfamiliar species that have a different ecological adaptation; and third, the genetic diversity of these species is mostly found in the tropics. With respect to these aspects, research and training centers like CATIE, which are located in the tropics, can play a decisive role in the development of research using biotechnology applied to tropical species.

Biotechnology Research in Coffee

Coffee is the major export crop of the CATIE mandate region. The emergence of new destructive pests and diseases, such as coffee rust, coffee borers, and nematodes, has reduced yield and increased the cost of production. Until recently, genetic improvement of cof-

fee needed 25 to 30 years to obtain isogenic varieties. In recent years, however, tissue culture techniques have opened important perspectives in research and practical applications for solving agronomic problems. In the Biotechnology Unit of CATIE, in coordination with IICA, two lines of research have been followed.

1. Somatic embryogenesis

2. Use of microstakes for the multiplication of superior genotypes

Somatic embryogenesis: The first investigator who succeeded in obtaining somatic embryogenesis in coffee was Staritsky (1970), using, as explants, orthotropic young stems of *Coffea canephora*. Herman and Haas (1975) and later Sondahl and Sharp (1977) were able to produce somatic embryogenesis in *Coffea arabica* using, as explants, fragments of young leaves. The latter authors found two types of response in embryogenesis: direct somatic embryogenesis, without an intermediate callus formation stage and embryogenesis induced from callus cells. From the viewpoint of multiplication, direct somatic embryogenesis permits the differentiation of 40 to 50 embryos from an explant of 1 cm^2 while with an intermediate callus step, the quantity of mature embryos obtainable reaches 200.

In CATIE's laboratory, results have been obtained using both processes, on different varieties. Nevertheless, the methodology has not yet been completely refined since the level of production of somatic embryos varies drastically with the environmental conditions in which the parent plants are grown. While the results with somatic embryogenesis using leaf fragments represents an important alternative in the massive multiplication of desirable genotypes, this method requires further study. On the other hand, other problems can be resolved more readily using different methods.

Use of microstakes for the multiplication of superior genotypes. This methodology is widely used in CATIE's laboratory to induce the development of lateral buds. There are three steps to the procedure:

1. Collection of orthotropic stems and their *in vitro* cultivation to establish an explant bank.

2. Clonal multiplication subcultivating the developed buds.

3. Rooting and acclimatization of the plants produced *in vitro*.

The results in our laboratory show the feasibility of the method, so long as certain factors are regulated:

1. Strict control of the explants to avoid bacterial and fungal contamination.

2. Adequate use of antioxidants to reduce polyphenols.

Experimental results at CATIE show that once the explants are available, 7 to 9 microstakes can be produced every 80 days. Use of the Caturra cultivar in this system has actually resulted in production of 20,000 to 25,000 plants per year. Acclimatization of these plants has been carried out in the greenhouse with no major problems and a 90% survival rate.

Since 1986, the laboratory has produced about 100,000 plants, of which approximately 60,000 have been grown in the field and sent to member countries of CATIE. The first experiments with the material produced from micropropagules were established in Costa Rica in 1987 and should produce their first crop by the end of 1988. So far, the coffee plants produced using this technology have shown no genetic alteration and their behavior and development has been similar to the parent plants.

Recently, another line of research was initiated at CATIE using micrografts. The objective of this research is to study new alternatives for nematode control, using a resistant genotype such as the robust species as a stock and the Catimor arabica clone, which is resistant to rust, as a graft. Very preliminary results show that there is compatibility between the two species and as soon as the technical problems of handling the micrografts *in vitro* are resolved, this methodology could be employed for the large-scale production of highly productive individuals resistant to both the major diseases and nematode pests.

Biotechnology Research in Cocoa

Cocoa has become a very important crop for the economy of Central America and other tropical countries. Indeed, this crop is a good example of a major agricultural product that is produced by developing nations in the tropics and consumed largely in the industrialized countries (Lass and Wood, 1985). The seeds of beans of *Theobroma cacao* provide the raw material for the manufacture of chocolate and for the extraction of cocoa butter.

In many ways, cocoa is an ideal crop for the small farmers with limited resources who constitute the principal target of CATIE's activities. However, cocoa suffers greater losses from a number of serious pests and diseases than many other tropical tree crops. According to Lass and Woods (1985), solutions to some of the

major problems are likely to come through further breeding and selection. Sources of new germplasm still exist; but the collection, evaluation, and maintenance of this material is a major task which is only now being addressed in an international context and to a lesser degree by the Regional Program in the various producing countries. At present, genetic improvement of cocoa is mainly based on exploitation of the heterosis that occurs in hybrids between the upper Amazon types and the amelonado or trinitario genotypes. This relatively simple cross of strong interpopulation heterosis provides the basis for almost all modern cocoa breeding programs (Kennedy *et al.*, 1987)

In the CATIE Biotechnology Unit, tissue culture methods are being employed in genetic improvement of cocoa: by developing haploid plants, by implementing a method of micropropagation, by using organogenesis and especially embryogenesis, and by studying methods that will permit the storage and free interchange of cocoa germplasm using simple methods of *in vitro* conservation.

Development of haploid cocoa plants. Dublin (1972, 1973) found that it was possible to obtain haploid plants spontaneously from monoembryonic and polyembryonic seeds. Our experience in the field has indicated that even when the haploid plants occur with low frequency, it is possible to identify haploid seeds by their flat shape. Using the CATIE germplasm bank and the CATIE method of producing hybrid seed, we separated flattened seed and found an adequate culture medium for the development of haploid embryos. It has been possible to isolate and germinate embryos that appear to be haploid under *in vitro* conditions. This material has been the source of explants for morphological studies with the aim of finding ways to stimulate organogenesis and especially embryogenesis. A high proportion of cytokinins with respect to auxins in the culture medium permitted the development of adventitious embryos from cotyledonary explants. It has also been possible to develop adventitious sprouts from lateral buds, all these coming from juvenile tissues.

Plants that appear to be haploid show marked morphological differences. We are intensifying our studies to confirm the level of ploidy in plants, and in tissue culture, and then to determine their point of origin. According to Laneud (1987), there are four types of spontaneous haploids in cocoa: from the female parent (Type A); from the male parent (Type B); those formed from a chimera of male and female parent tissue (Type C); and haploid-diploid chimeric parts in which the diploid part is a heterozygote (Type D). We have not yet clarified the origin of our *in vitro* cocoa plants; nevertheless, these plants show the characteristic morphology of

haploid plants: small leaves, special veining patterns, and small plant size.

The studies with these presumed haploids may lead to obtaining isogenic lines following duplication of the genome with colchicine and to the development of an efficient means of clonal propagation of those superior individuals, either by tissue culture or by conventional methods of cloning and grafting.

Micropropagation. We are studying possible techniques of massive propagation using somatic embryogenesis and *de novo* organogenesis. In the first case, we have found that it is possible to develop callus of juvenile tissue, both haploid and diploid. In both cases, the callus emerge from the surface of the cotyledons or from the meristematic apices of plants that germinated a few days earlier.

The white callus shows differences after about forty days: embryogenic structures are observed which take considerable time in developing. We have found that from veins of the cotyledons, somatic embryos are similarly differentiated, apparently without passing through the callus stage. Preliminary studies utilizing media of different pH show that both callus formation and somatic embryogenesis are increased through the use of more alkaline pH, as by using culture media with a pH above 6.7.

A system of micropropagation of microstakes derived from adult tissues is being used. The major limiting factor in this method is to adequately disinfect plants that generally come from the field.

Conservation and Exchange of Cocoa Germplasm. The exchange of germplasm between countries is considerably limited by phytosanitary problems. Furthermore, the viability of cocoa seed is of short duration, making exchange of germplasm by means of seed especially difficult. Even adult vegetative material has shown a short storage life. For this reason, we have begun studies for the maintenance and exchange of germplasm *in vitro* coming from seed, principally of self-compatible individuals. The seed is germinated *in vitro* and the resulting plants maintained for periods greater than four months. The use of growth retardants is being studied as well as the use of liquid media for transport and culturing in the absence of light. Material germinated *in vitro* has remained viable for more than four months, a period that we consider adequate for exchange of genotypes among countries. In the future, we are contemplating the use of micrografts for grafting adult buds into juvenile material *in vitro*.

The development of efficient micropropagation is vital to the management of genetic resources of cocoa. A rapid, inexpensive micropropagation technique would have great impact on the economics of germplasm multiplication and distribution. *In vitro* techniques for collecting cocoa germplasm from the wild will also make a major contribution to genetic conservation (Yidana *et al.*, 1987).

It is our feeling that the present research in biotechnology for cocoa is not yet sophisticated enough to use techniques such as recombinant DNA. Further advances in our knowledge of organogenesis and embryogenesis *in vitro* are needed to develop efficient systems of regeneration of plants by any process. Advances in tissue culture methods do permit the use of micropropagation for cloning desirable genotypes at the present time. It will also be possible in the near future to conserve genotype *in vitro*. Long-term expectations include the use of genetic engineering in cocoa for genetic improvement, obtaining resistance to disease, and finally conserving germplasm indefinitely through the use of cryopreservation.

Biotechnology Research in Plantain

Unfortunately, there is relatively little information about the role of the Musaceas (bananas and plantains) in the diet of the inhabitants of most tropical countries. Possibly for this reason, little effort has been made with these crops, resulting in considerable gaps in the technology available to work with them.

Jaramillo (1987) estimates that world production of Musaceas is more than 60 million tons of which about 20 million are produced in Latin America and the Caribbean, occupying an area of some 1.5 million hectares. Of this production, about one seventh, with a total value of some 1,100 million dollars, is exported. Plantain exports account for approximately 600 million dollars. These figures should give some idea of the great importance of the plantain, indicating how urgently the Musaceas need greater scientific attention.

As was indicated previously, CATIE has certain advantages for carrying out research with Musaceas, especially plantain. Research in plantain will emphasize the production of clones that are tolerant or resistant to plantain diseases and identification of biological control methods for black sigatoka, the major disease of the crop. To attain these objectives, tissue culture techniques are being used to obtain somaclonal variation and micropropagation of individuals that show desirable characteristics, principally resistance to disease. Different "variants" have been obtained and are being

planted in the lowland experiment station of CATIE, "La Lola," to be evaluated in the field under high selection pressure. A concurrent program micropropagates genotypes that have been selected in the field for disease resistance. The present capacity of CATIE's laboratories permits the production of more than 400,000 plantain plants per year without greatly affecting the continuity of other research projects.

The plantain varieties presently under study are of the Horn plantain (AAB), locally known as "Currare," and of the French plantain (AAB), locally known as "Dominico," groups. The micropropagation system developed in the CATIE laboratory has proved to be quite efficient, permitting the initiation of plantings this year to evaluate the performance of genotypes presently found in our germplasm bank.

The International Network for the Improvement of Banana and Plantain (INIBAP) was established in November 1984 by a group of donor countries and organizations with the general mandate to improve Musas. Under an agreement between CATIE and INIBAP, the regional office of INIBAP was established at the CATIE headquarters in Turrialba, Costa Rica and CATIE assumed responsibility for receiving and maintaining the world reference collection for Musas. To properly fulfill this responsibility, CATIE is assessing the comparative adequacy and security of conserving this important germplasm. Economic support has been requested from IBPGR to develop techniques of cryoconservation in the Musas. The material that is received now and in the near future will be stored *in vitro*.

In order to intensify basic research in the Musas, exchange agreements have been signed with several research centers in France. As a result, CIRAD in Montpelier has agreed to send French research personnel to work in coordination with CATIE researchers on the pathology and genetics of Musas, using biotechnological tools. In preparation for this collaboration, CATIE researchers have received training in cryoconservation and other laboratory methods in the CNRS laboratory in Meudon, France.

Biotechnology Research in Promising Tropical Crops

The tropics have a tremendous potential for human food production. The possibility for year-round production and the presence of greater biological diversity are only two of the comparative advantages upon which this potential is based. The lack of research attention to tropical crops, however, is an obstacle to realizing the possible productivity. Agricultural research has concentrated on less than 20 edible species and has emphasized production in the temperate

zones. The development of greater crop diversity will depend on whether the tropics can preserve their biological diversity as a genetic bank for species improvement and the appropriate technologies for germplasm utilization and preservation can be developed.

A number of promising crops have the potential to contribute to the economy of the Central American region through agricultural diversification. These crops fall into four categories: 1. roots and tubers that have great potential as human food; 2. fruit trees; 3. spices and dyes; and 4. ornamentals. Until now, biotechnology methods have been applied to root and tuber species for germplasm conservation and for the elimination of viruses. The principal objective of research on ornamentals is micropropagation of orchids in danger of extinction. The use of biotechnology in tropical root and tuber crops will be discussed.

The principal tropical root and tuber species are cassava (*Manihot esculenta*), sweet potato (*Ipomoea batatas*, coco yam (*Xanthosoma* spp.), true yam (*Dioscorea* spp.), taro (*Colocasia* spp.), and some others of local importance. The genetic diversity of these species has been studied in Central America as well as in other tropical regions. CATIE now has large living collections of many genotypes of these species. There is considerable risk involved in maintaining germplasm in field collections as they are exposed to epidemics, environmental catastrophes, and human error. Maintaining living collections in the field is also extremely expensive, due to the large amount of labor and other inputs required. It was therefore considered expedient to establish *in vitro* germplasm banks for this material. Presently, at least six clones of every introduction are being maintained *in vitro* (Table 1). Depending on the species, these materials are transferred to fresh media every six to twelve months. As this transfer of germplasm from field collections to *in vitro* conditions occurs, studies are carried out to reduce the frequency of transplants needed and to consider the use of cryopreservation. The use of growth retardants appears to be a good method of reducing the need for such frequent transplants. Several such materials, including aspirin (Acido acetil salicato) have been tested in sweet potatoes. The results were very satisfactory with reductions of up to 100% in the growth rates being found. Field experiments indicate that after exposure to acido acetil salicilate for various periods, plants recover their normal growth without evidence of phenotypic changes. Current morphological and anatomical studies on this material emphasize stomatal behavior. At the same time, biochemical analyses are determining proteic and enzymatic profiles associated with respiration. The studies with salicylic acid are being extended to other species of roots and tubers that have similar problems.

Table 1. Root and Tuber Species Maintained in the *In Vitro* Germplasm Bank.

Genus	Species	Common Name	Number of Introductions
Colocasia	esculenta	ñampí	1
Colocasia	sp	ñampí	9
Dioscorea	alata	ñame	5
Dioscorea	dumetorum	ñame	1
Dioscorea	sp	ñame	11
Dioscorea	trifida	ñame	7
Ipomoea	batatas	camote	67
Manihot	esculentum	yuca	78
Xanthosoma	nigrum	tiquisque	2
Xanthosoma	sagittifolium	tiquisque	3
Xanthosoma	sp	tiquisque	18

Economic support has been requested of IBPGR to begin research in cryoconservation. Although cryoconservation techniques will be applied first to the crops given priority in the program, they may later be applied to root and tuber crops.

Parallel to germplasm conservation, programs are being implemented to obtain virus-free root and tuber plants. The objective is to develop efficient micropropagation systems that can provide virus-free planting material to farmers in CATIE's member countries. Hopefully, a program currently being developed in Costa Rica eventually can be extended to the other member countries.

Training in Biotechnology

CATIE is regarded as the most important center in agricultural education in the Central American isthmus and the Caribbean. During the forty-year existence of the graduate program, over one thousand Masters degrees have been awarded to students from all countries in the Western Hemisphere. Each year, CATIE receives 250 to 300 applications for the M.S. program not only from Latin America, but also from the Caribbean, Canada, the United States, Asia, and Europe.

The role of CATIE in enhancing human resources is vital for the development and modernization of agriculture in the region. While this training is directed to produce a primary impact in Central America and the Caribbean, CATIE's educational mandate covers all countries of Latin America. The Center does not limit its training

activities to the Masters degree program and a significant portion of the educational activity focusses on training agricultural technicians through refresher and in-service courses.

Each year, the program of tropical crop improvement trains more than 250 technicians at different levels and in a variety of specialties. In the area of biotechnology, for example, two courses were given in 1987, one at the Masters degree level and an international training course whith participants from fourteen Latin American countries. At the same time, in-service training in tissue culture techniques was given to 20 researchers from Latin America and Europe. Based on the applications for admission received for 1988, teaching and training activities should be even greater this year.

Cooperative training and teaching arrangements. In order to support biotechnology development in Latin American countries, an international course was organized at CATIE in collaboration with Colorado State University (CSU). Arranged through CSU's Tissue Culture for Crops Project (TCCP), the course familiarized 22 scientists from 14 Latin American countries with *in vitro* techniques applied to important agricultural and forestry species in the tropics. This course, partially financed by USAID, stimulated great interest in agricultural biotechnology in the countries involved. With the support of Colorado State University through the International Plant Biotechnology Network (IPNet), this course will be given again in 1988.

CATIE offers many advantages for teaching such courses because of its strategic location, close involvement with regional problems, modern facilities, and training materials in the Spanish language. A similar approach will be used in a course in genetic engineering to be given in 1989 in collaboration with the Johann Wolfgang Goethe University of Frankfurt, West Germany. The course will be offered to scientists in the region who are interested in developing research in the area of cellular transformations. Other educational activities have included intensive training of young researchers in the field of biotechnology in Guatemala, the Dominican Republic, and Costa Rica. In future years, this activity will continue to give great impetus and support to national programs in biotechnology that are emerging throughout Central America.

Research Agreements and Commercial Arrangements

The application of biotechnology to agricultural crops has great economic potential for CATIE's member countries. This importance is being recognized not only by government programs but also by

the private sector. The great number of requests for training received by the Biotechnology Unit is evidence of this interest. At the same time, agreements have been reached for the sale of plants produced by tissue culture, principally with Costa Rican enterprises. The principal interest is the purchase of ornamental plants cloned by micropropagation. Similar material should be available in plantains, cocoa, and bananas at a later date.

The Biotechnology Unit of CATIE is applying biotechnological tools to the solution of specific problems in the agriculture of the member countries. For example, under an agreement with the University de Valle in Guatemala, virus-free cardamom (*Eleittaria cardamomum*) plants will be obtained through meristem culture. Within the guidelines of this agreement, CATIE is developing technology for obtaining healthy germplasm. That technology will then be transferred to and used by the University del Valle. Another agreement has been signed with the Ministry of Agriculture of the Dominican Republic for assistance in the establishment, training, and donation of *in vitro* germplasm that will permit the massive multiplication of Musas, roots, and tubers (cassava, yams, sweet potatoes, and others). This agreement resulted in the reactivation of a tissue culture laboratory, the training of technical personnel, and the initial production of plants obtained through micropropagation techniques.

In Costa Rica, CATIE signed an agreement with the Ministry of Science and Technology to implement a national program for obtaining basic seed free of virus in four species: cassava, yams, taro, and cocoyams. As was pointed out earlier, the roots and tubers are of great importance as a source of nutrients in the region and have considerable export potential as well. Through this agreement with the government of Costa Rica, CATIE hopes to establish a program of basic seed production that includes the whole process from meristem isolation to the delivery of planting material to the farmer. The participation of both the Ministries of Agriculture and of Science and Technology is contemplated as well as that of the private sector and medium– and large-scale producers. Although such interdisciplinary activities are complex and require much commitment from the participating individuals and agencies, it is hoped that 25 demonstration plots will be established in representative regions of the Central American isthmus. In these plots, virus-free plants obtained by *in vitro* techniques will be compared with plants multiplied by farmers using traditional methods.

Farmers who grow ornamental species for export have shown great interest in the use of tissue culture techniques for cloning selected individuals. An agreement is presently under study with a private

flower-exporting firm in which CATIE will develop the technology and multiply selected genotypes of shade plants, orchids, and other exotic species. This type of agreement will open the door to self-financing activities and may even help CATIE to generate some resources for research endeavors.

Areas of Collaboration

Biotechnology clearly is playing an important role in CATIE's activities, a role that will intensify in the near future. The urgent need to increase yields and produce genotypes resistant to diseases in the coffee, cocoa, and plantain crops of the region cannot be ignored. Thus far, real advances in biotechnology in these crops have been modest, despite their importance to the region, compared to the progress made in many temperate crops. For this reason, tissue culture studies must be intensified in order to understand the morphological events that precede micropropagation (organogenesis and embryogenesis). Similarly, genetic studies must be increased in the areas of somaclonal variation, obtaining haploid plants, wide crosses, and cellular transformations.

CATIE's extensive germplasm bank of the priority species creates a very advantageous position, with respect to other centers. This advantage, however, is somewhat limited by the lack of personnel and resources to carry out adequate and timely evaluation of this germplasm. We believe that studies of morphogenic conditions should be intensified in order to arrive at an efficient system of micropropagation of cocoa. Genetic variability in the Musas must be increased; while coffee must be improved with respect to resistance to nematodes, borers, and rust. In order to fulfill these objectives, interdisciplinary research teams and economic support are urgently needed. It is in this area that collaboration is most essential.

Economically promising crops are another important component on the biotechnological horizon. For this purpose, as well, the importance of a center with large germplasm banks cannot be overestimated. Even with this advantage, very few studies have been made of the tissue culture methods for species such as black pepper, vanilla, cardamom, tropical fruits, roots and tubers, ornamentals. Our strategy is to develop technological packages and to be able to multiply superior germplasm through tissue culture techniques. For this reason, collaboration is also sought from commercial enterprises interested in supporting research with such species and researchers who are interested in working with these species.

A central necessity for CATIE's activities in biotechnology is, without a doubt, the intensive study of all of the possible options for preserving germplasm banks with maximum stability for the longest period at the lowest cost, especially for species propagated organically. This activity is of the highest priority in the application of biotechnology to tropical species. In this respect, CATIE has received considerable support from the IBPGR and the government of the Federal Republic of Germany. Because adequate maintenance and use of these genetic resources requires the combined effort of many research institutions and the private sector, this is the third area of priority for collaborative activities.

Finally, the training and teaching component should be considered as a major priority for inter-institutional activity. The agreements with the Johann Wolfgang Goethe University and with Colorado State University advance this objective. Efforts should be made to increase collaboration in biotechnology education and training for scientists and technicians from the countries of Latin America and the Caribbean.

Future Goals

CATIE's program in the improvement of tropical crops proposes four long-term goals:

1. Improvement of coffee, cocoa, and plantain with the objective of obtaining more productive and disease-resistant planting material.

2. Collection, maintenance, evaluation, and distribution of promising tropical genetic resources.

3. Technology development through emphasizing research on critical components of perennial and promising tropical crops.

4. Teaching and training in modern techniques of biotechnology applied to tropical species.

To obtain these goals, special emphasis must be given to biotechnological tools that can be used in combination with conventional breeding and agronomic methods to obtain solutions as rapidly as possible to production problems in coffee, cocoa, plantains, and other promising crops for the Central American region.

In the near future, CATIE will also promote positive interaction with other research institutions and with the private sector to initiate research into new technologies to increase agricultural production.

In this respect, it is important to recognize the enormous potential of plant-microbe interactions that can play a decisive role in achieving the goals of the program.

Another area of interest to CATIE is the implementation of methods of biological control of foliar diseases in cocoa, plantain, and coffee which are presently controlled through the use of pesticides at high cost. Preliminary research conducted in the control of moniliasis of cocoa and black sigatoka of plantain through the use of antagonistic bacteria has had positive results.

We are primarily interested in collaborating with institutions and enterprises that are actively working with genetic engineering approaches using microorganisms in the areas of nitrogen fixation and biological control. Our goal is to adopt some of the new technologies to existing activities at CATIE and to conduct joint research efforts on ecological studies that may unravel the complex interactions and, thus, may determine the success of this approach.

Finally, it is important to emphasize that the advantages of modern biotechnological techniques now emerging can most constructively be used in tropical countries to solve some of the complicated problems that face tropical crop species. To use these techniques, an interdisciplinary approach is required from both the private and public sector. Without this combination of efforts, the advances that seem possible over the short and medium term may not be realized for a long time to come. During such a period of dubious effort, genetic resources presently available may be permanently lost.

References

Dublin, P. 1972. Polyembryony and haploidy in *Theobroma cacao*. Cafe, Cacao Thé 16:295-311.

Dublin, P. 1973. On the use of genetic marker in the search for haploids in cocoa tree (*Theobroma cacao*) L.). Cafe, Cacao Thé 17:205-210.

Gupta, P.K., S.V. Kendurkar, V.M. Kulkarni, M.V. Shirgurkar, and A.F. Mascarenhas. 1984. Somatic embryogenesis and plants from zygotic embryos of coconut (*Cocos nucifera*) *in vitro*. Plant Cell Reports: 222-225.

Herman, F.R.P. and G.J. Haas. 1975. Clonal propagation of *Coffea arabica* L. from callus culture. Hortc. Sci.:10:588-589.

Jaramillo, R. 1987. Memoria de la Reunión Regional de INIBAP para América Latina y El Caribe. San José, Costa Rica. pp. 284.

Kennedy, A.J., G. Lackwood, G. Mosso, N.W. Simmonds and G.J. Tam. 1987. Cocoa Growers' Bulletin. 38:5-22.

Lanaud, L. 1987. Nouvelles données sur la biologie du cacaoyer (*Theobroma cacao.*): diversité des papulations, systeme d' incompatibilité, haploides spontanes, levrs consequences pour l'amelioration de genetique de cette espece.

Lass, R.A. and G.A.R. Woods. 1985. Cocoa production. Present constraints and priorities for research. World Bank Technical Paper Number 39. Washington, D.C. 95 pp.

Litz, R.E., R.J. Knight and S. Gazit. 1984. *In vitro* somatic embryogenesis from *Rangifera indica* L. callus. Scientia Horticulturae, 22:233-240.

Roca, W., M.C. Amezquita and V.M. Villalobos. 1986. Estado actual y perspectivas de la biotecnología agrícola en Amaérica Latina y El Caribe. Encuesta 1986. CIAT, pp. 39.

Staritsky, G. 1970. Embryoid formation in callus cultures of coffee. Acta Bot. Neerl. 19:509-514.

Sondahl, M.R. and W.R. Sharp. 1977. High frequency induction of somatic embryos in cultured leaf explants of *Coffea arabica* L. Z. Pflanzenphysiol. 81:395-408.

Withers, L.A. 1984. Working group of cocoa, IBPGR (1984). Report of the second meeting, Arlington, Va. USA. IBPGR, Rome.

Yeh, M.L. and W.CH. Chang. 1986. Plant regeneration through somatic embryogenesis in callus culture of green bamboo (*Bambusa oldhamii* Munro). Theor. Appl. Genet. 161-163.

Yidana, J.A., L.A. Withers, and J.D. Irins. 1987. Development of a simple method for collecting and propagating cocoa germplasm *in vitro*. Acta Hirticulturae 212:95-98.

Use of Cell Genetics for Crop Improvement

M.R. Söndahl and D.A. Evans
DNA Plant Technology Corporation
Cinnaminson, New Jersey

Selecting Technology

Successful commercialization of plant biotechnology requires the application of a wide spectrum of technologies, ranging from modern forms of plant breeding (plant level) to cellular genetics and molecular biology. Careful selection and integration of such technologies are key elements for development of new products. Depending on the project goal, very simple and short-term technologies could be applied; whereas, in other cases, only medium- to long-term technologies will deliver new products. It is very critical to analyze the feasibility vs. costs and timetable to completion before undertaking a project. Such a risk/benefit assessment could result in recommendation of the use of different technologies with different consequences regarding the final completion of a product and its total development costs.

A balanced portfolio of short-, middle-, and long-term projects would bring maximum benefits to most biotechnology companies. Micropropagation coupled with disease-free stocks can offer immediate paybacks. In cases of high costs of micropropagation units, this technology should be applied in conjunction with standard horticulture and/or breeding methods.

Somaclonal and gametoclonal variation are cellular methods that exploit natural variation within plant cells. Such a program begins with removal of cells from the best plant varieties or hybrids, recovers regenerated plants, and selects new variants under greenhouse and field conditions. Depending on the species, vegetative or seed propagation schemes have been established to ascertain the stability of new traits during subsequent generations and under different environmental conditions. Such techniques allow the development of new plant varieties in about half the time required using conventional methods. Within cellular genetics, the production of homozygous lines from F_1 hybrid or highly heterozygous materials is accomplished by recovering double haploid plants derived from the culture of gamete cells (pollen grains or ovules). In contrast with the need for 6-8 successive generations of selfing, this gamete culture technology can deliver new inbreds within one cycle (less than one year for annual crops). Distant crosses can be obtained by embryo rescue techniques,

whereby immature hybrid embryos are recovered under culture conditions. Protoplast fusion methods permit access to new plant hybrids, but perhaps the most interesting advantage of this technology is to allow organelle transfer between such fusion products. Plants derived from embryo rescue or protoplast fusion techniques must undergo standard breeding procedures to recover commercial varieties.

Molecular biology is enlarging our knowledge of the complex genetic structure and control of gene expression at the cell, tissue, and whole plant level. Recombinant DNA technology offers the ultimate tool to transfer specific genes to develop new plant varieties or hybrids. Although this approach has a much longer timeline than cellular genetics, a balanced level of activity in this area will assure future success where plant breeding and cell genetics would be limited.

Another area of interfacing technologies is in the development of plant disease diagnostic kits using hybridoma technology for the production of monoclonal antibody reagents specific for certain plant or soil pathogens.

Programs on development of new plant varieties that focus on processing and/or consumer benefits would also require expertise in the area of food technology to successfully screen new value-added products. Similarly, if the R+D goal is directed to the agrochemical industry, additional activities, such as chemical registration, have to be added to permit a successful completion of the program.

Once the new plant variety or hybrid is produced, it will require additional expertise to first produce test-market or pilot-plant quantities, followed by a large-scale production scheme.

Defining Strategy

It is imperative that short-, middle-, and long-term strategies are defined in order to guide decisions and thus enhance the probability of success. A private plant biotechnology company has to devote its efforts to generation of significant revenues to pay for R+D expenses, costs of market introduction, and to ensure a reward to investors. In addition, it should focus on specific market areas and develop maximum technological leadership. Private companies could focus on variety improvement to enhance farming productivity and provide new value-added products for the processing industry or final consumers. Whatever the position of the biotech product, the activities should be consistent with the overall strategy for product commercialization, i.e., directed to seed sales designed

for the processing industry, or focused on supermarket sales. Sensitivity to changing trends and values of modern society would help the success of future products. It is necessary to focus on early product introduction, development of a constant flow of new products, and development of new technologies to insure a long-term survival. An active patent strategy will secure a proprietary portfolio of products and technologies for commercialization and future developments.

Selected DNAP Products

DNA Plant Technology Corporation, (DNAP), focuses on the development of new plant varieties with value-added to the final consumer and industrial markets.

One opportunity DNAP has identified is to develop new improved produce. Little, it seems, has changed in the produce section of the supermarket over the past several years. A tomato is still a tomato, although its taste and texture might be different at different times of the year. A carrot is still a carrot. At times it might be juicy and flavorful and at other times its taste and texture might be disappointing. It is a rare occasion today when the consumer can reach for a brand name of produce and be assured of an eating experience that measures up to his or her expectations day in, day out, week after week, and month after month. All of this is about to change. It is now becoming possible to offer products that are still 100% natural, have the same nutritional content of current varieties and yet have a better taste, a better texture, a more enjoyable aroma and, importantly, offer these benefits consistently, year round. The technologies are available for producing new varieties of vegetables and fruits that will offer superior values to the consumer and that can be branded for easy recognition. This revolution in technology is taking place at the same time that consumers are becoming increasingly interested in good health and nutrition and are looking to satisfy these needs with fresh, natural produce and fruit items. DNAP is focusing on applying these new technologies to develop new and improved varieties of agricultural products to meet this consumer demand.

A. VegiSnax

VegiSnax[R] vegetables consist of a whole line of superior-tasting fresh vegetables, pre-cut and packaged, available all year in supermarket and convenience stores. DNAP has been test-marketing VegiSnax[R] carrots and celery. The development of VegiSnax[R] vegetables reflects the approach we are taking in the whole area of branded produce. We began with the consumer. We asked con-

sumers, in focus groups, to identify for us the qualities of the ideal carrot and the ideal celery. From these focus groups we developed a standard which became our product objectives. We began by searching the globe for the best varieties in existence. Our plant breeders worked to optimize these varieties. Then we began to develop varieties that would improve on these through the use of cell genetics and plant breeding. Market projection for this product line in the United States is estimated to be $100 million a year.

B. Fresh Market Tomato

Our work in the area of fresh market tomatoes is proceeding along the same lines as our VegiSnax[R] branded vegetable strategy. Consumers would like to enjoy all year round the taste of a vine-ripened tomato picked at the height of the growing season. We searched for the best available varieties, and we have been applying cell genetics technology to develop new varieties that will meet the standard consumers are looking for. The market for fresh tomatoes in the United States is approximately $500 million annually, at retail.

C. Rice

Rice is the second largest crop produced in the world today, and rice products account for close to $750 million of sales annually in U.S. supermarkets alone. The application of biotechnology to develop new proprietary hybrid rice varieties and new rice-based food products for the consumer market represents a major commercial opportunity.

D. Popcorn

Popcorn is one of America's most popular snacks. The total U.S. popcorn market is $1.2 billion a year. The U.S. market for microwave popcorn amounts to about $350 million annually. Popcorn is a healthy snack, but most people typically add salt and butter to improve taste. If, for example, a better-tasting popcorn could be developed, it would allow consumers to enjoy popcorn without "additives." DNAP is using both modern forms of classical plant breeding and somaclonal variation to develop superior varieties of popcorn.

E. Natural Sweeteners

Several plant-based sweeteners exhibit sweetening power many times that of sugar and can be used effectively to sweeten low-calorie food and beverage products. While the existence of such

compounds has long been known, the "productivity" of plants producing them is currently too low to make them commercially viable on a large scale. DNAP is applying various cell culture techniques to develop improved varieties that will produce greater quantities of these natural sweeteners. One current advantage of such natural sweeteners will be the heat stability during cooking and baking.

F. Flavor and Fragrances

Plants are the principal source for many compounds used in the flavor and fragrance industry. Improving yields or enhancing the plant's adaptability to different growing environments can significantly reduce costs, improve the quality, and add to the reliability of the supply of many key flavor and fragrance compounds. DNAP is applying somaclonal and gametoclonal variation technology to develop improved varieties of plants producing commercially important flavor and fragrance compounds.

G. Processing Tomato

The food processor—the maker of tomato sauce, paste or ketchup—makes use of the solid portion of the tomato, not the liquid. The U.S. market for processing tomatoes is $500 million a year. To the extent that solids can be increased without changing flavor or nutritional qualities, considerable savings can be generated at the farm and the processor level. Through somaclonal variation technology, DNAP, starting with the 5% solids variety, has succeeded in developing a new variety that is approximately 20% higher in solids. DNAP was awarded a Plant Variety Protection Act Certificate by the U.S. Department of Agriculture for this new variety, the first ever awarded for a variety developed through somaclonal variation.

H. Tropical Plants

Coffee, cacao, banana, and citrus are just a few plantation crops produced in tropical and sub-tropical areas that enjoy very large markets in the United States (ca. $12 billion a year) and other temperate countries. Coffee and cacao are two major tropical plants that DNAP has targeted in its product development program. The processing of coffee beans and cacao beans, depending on the end product, can involve a number of steps that will affect quality and cost. Traditionally, food companies have sought to improve quality, lower costs, and add value to the product delivered to the consumer by process improvements at the manufacturing facility. Advances in plant tissue culture and cellular genetics now make it

possible to create processing efficiencies and add value to the product at the raw material stage by developing new, improved plant varieties.

I. Plant Diagnostic Kits

Early detection of plant diseases can enable lawn care specialists, growers, and nurserymen to intervene effectively before serious damage has occurred. Detection of plant diseases is often a time-consuming, costly process, typically involving delivery of a sample of the infected plant material to the plant pathology laboratory. An inexpensive detection system that can be used by a layman in the field represents an important commercial opportunity. DNAP, through its Agri-Diagnostics Associates' joint venture with Koppers Company, has developed monoclonal antibody-based detection kits for fungal diseases affecting turf grass. These kits are now being test-marketed to golf courses, with future testing planned for lawn-care professionals. The development of detection kits for diseases affecting a major commercial row crop, is now under way. Other applications of monoclonal antibody technology, including use for the detection of chemical residues in soil, are also targets for Agri-Diagnostics Associates.

Strengthening Collaboration in Biotechnology

Public and private biotechnology institutions have important and distinct MISSIONS to society. National and international research institutions should have primary responsibility in the areas of high-risk technology development, personnel training, supporting data for new legislation, recommending guidelines, and monitoring safety of rDNA experiments. Private companies have a primary mission to generate and commercialize products, operate within a profit margin that compensates the capital of public investors, and at the same time provides jobs and pays taxes to the society. Efforts to make profits at public institutions or to have non-profitable activities in the private sector would distort these missions and most frequently lead to complete failure.

Every institution should recognize WHAT ARE THE BIOTECH PRODUCTS within the scope of its activity: (a) technology (patents; licensing and royalties); (b) seeds; (c) proprietary products (flowers, etc.); (d) new varieties or hybrids; (g) gene constructions, etc.

In addition, new biotech products and discoveries have to be recognized and treated as any other INVENTION produced by mankind, if full benefits are to be returned to our society. To accomplish that, adequate patent legislation should be in existence.

All institutions engaged in biotechnology should make a fair ASSESSMENT OF THE VALUE of each product and thus be in the position to enter into either a commercialization activity or licensing agreements.

Any technology or product developed by the public sector could be immediately commercialized by the private sector under exclusive or non-exclusive licensing agreements, provided that proprietary patent protection has been secured. It is important that national and international institutions have a system in place that permits such patent applications before public disclosures take place, and thus secure revenues from commercialization or eventual royalties to the institution and inventors. Lack of such a policy seems to be the most common factor leading to failure of collaborations between the private and public sectors. It is fundamental that private companies must guarantee proprietary protection or exclusive agreements in order to justify the long-term investment necessary to bring a product to test market and full-scale commercialization. A clear policy on this issue would facilitate tremendously the relationship between public and private sectors.

In other instances, research groups from both the private and public sectors could jointly develop products and technologies and have joint ownership of their discoveries.

In summary, we firmly believe that once legislation to protect all discoveries of biotech research are in place and adequate policies are implemented in order to deal with the proprietary nature of R+D discoveries, collaboration between public and private sectors will be fostered to a greater extent.

Tissue Culture Micropropagation and CIAT Biotechnology Research

W.M. Roca* and B. Nolt**
Biotechnology Research Unit* and Cassava Program**
CIAT Cali, Colombia

Introduction

Root and tuber crops such as cassava, potatoes, sweet potatoes, yams, taro, and the Andean tubers are preferentially, or only, propagated by vegetative means and grown as clones. This mode of propagation often exposes these crops to pests and diseases that may be transmitted from one vegetative generation to the next, with the result that entire cultivars may become infected. Viral diseases, in particular, may remain latent, but crop yield and/or quality decline over time. Viruses and other systemic pathogens restrict the international exchange and conservation of crop genetic resources. Breeding for resistance to viruses is generally difficult in vegetatively propagated crops and does not allow rehabilitating cultivars with already valuable agronomic attributes; therefore, production of high-quality planting material constitutes a very important factor for good cv. performance.

Asexual propagation utilizing various plant parts, e.g., cuttings, stakes, tubers, corms, rhizomes, etc., has been developed into standard agricultural practice. In the last 10-15 years, asexual propagation methods have been extended to tissue culture techniques. Guided by specific agronomic objectives, tissue culture methodologies may (as much as possible) provide support to, or replace, some stages of the propagation process. A combination of tissue culture and traditional vegetative propagation techniques may be beneficial in one particular stage, while propagation by sexual means may be more appropriate at another stage for the same crop.

Micropropagation

Clonal propagation is currently the first important practical application of plant tissue culture. *In vitro* propagation techniques have received increased attention because: a) pathogens can be excluded from systematically infected plants and, thus, certified disease-free clones can be produced; b) only a small amount of space is required to multiply and maintain large numbers of plants; and c) rates of multiplication much greater than with traditional techniques can be obtained.

Micropropagation Methods

There are two possible routes for *in vitro* culture propagation: a) through enhancement of preexisting meristems, and b) through *de novo* formation of meristem primordia or somatic embryos. The former is the most common route of micropropagation. Since multiplication takes place by enhancing preformed axillary buds, genotypic stability is maximized. An important added advantage is the possibility of disease elimination. Although it is possible to geometrically multiply shoots, this route requires much labor to generate whole plants since shoots need to be individually rooted. Hence, *in vitro* propagation has major commercial application in the propagation of high-value plants, e.g., ornamentals, and the production of early generation disease-free transplants.

The second route of micropropagation utilizes explants which do not harbor any preformed meristem. Propagation takes place through direct or indirect organogenesis or somatic embryogenesis (Fig. 1); the latter pathway potentially offers very high multiplication rates. The occurrence of an intermediary callus in the indirect route may cause cytogenetic instability and somaclonal variation (Scowcroft, 1984). Because of their bipolar structure, somatic embryos do not require separate shoot growth and rooting steps (Bondaryk and Styer, 1985); furthermore, embryogenic cultures can be multiplied in suspension cultures to very high numbers; in this stage, such cultures may be amenable to bioreactor automation (Fig. 1), and production of artificial seeds by embryo encapsulation or production of synchronized embryo cultures for fluid drilling transplanting (Ammirato, 1983). Major needs to develop somatic embryogenesis propagation for important agricultural plants include: regeneration of somatic embryos, control of the process in suspension cultures, and improvement of current bioreactor limitations, i.e., doubling time, cell clustering, etc.

Disease Elimination

Disease elimination enables rehabilitating yield and quality of local cvs. Application of thermotherapy and/or chemotherapy to infected plants and cultures, in association with meristem-tip culture, generally increases the rate of pathogen exclusion (Roca, 1985). An inverse relationship exists between the size of the explant and the rate of virus elimination; highest rates are achieved with explants comprising the apical dome plus one or two of the youngest primordial leaves (Schilde-Rentschler and Roca, 1986). The technique is valid for all infectious agents, but is particularly important for viruses and virus-like agents. Pathogen elimination depends not

only on a good tissue culture technique, but also on reliable methods of disease indexing.

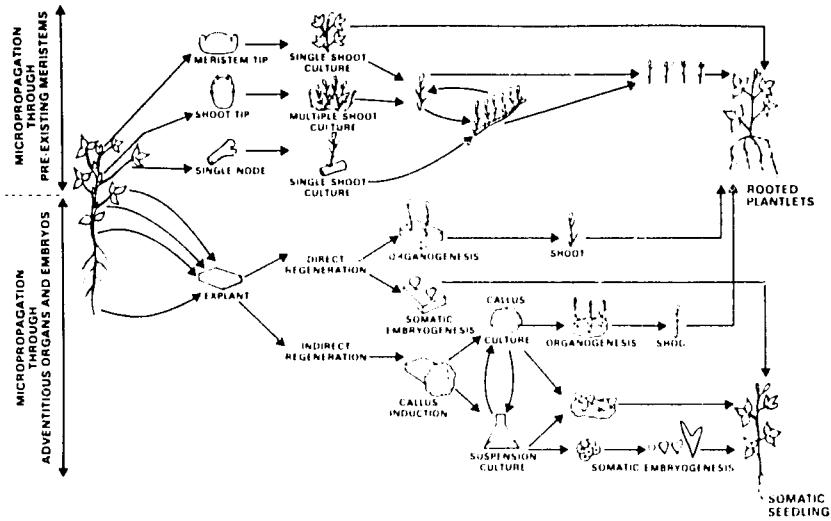


Figure 1. Possible routes of micropropagation: a) through enhancement of axillary buds using meristem tips, shoot tips and nodes, and b) through organogenesis and somatic embryogenesis using explants devoid of preformed meristems. While route a) favors trueness-to-type, somatic embryogenesis in liquid-shake culture provides the highest propagation potential. (Adapted after George and Sherrington, 1984).

Applications of Advanced Detection and Diagnostic Techniques to Viruses Infecting Cassava in Latin America

To date, five plant viruses and several virus-like diseases have been reported on cassava in Latin America. The relative importance of these agents to cassava production reflects their geographic distribution and severity.

Viruses	Origin	Distribution	Severity
Cassava Rhabdovirus	Brazil	Narrow	Low
Cassava Vein Mosaic Virus (CVMV)	Brazil	Intermediate	Medium
Cassava Common Mosaic Virus (CCMV)	Brazil	Wide	Medium
Cassava Colombian Symptomless Virus (CCSV)	Colombia	Narrow	Low
Cassava X Virus (CsXV)	Colombia	Intermediate	Low

Viruslike Diseases	Origin	Distribution	Severity
Caribbean Mosaic Disease (CMD)	Colombia	Narrow	High
Frogskin Disease (FSD)	Colombia	Intermediate	High
Latent Agents	Colombia	Wide	Medium
Cassava Aucuba Mosaic	Cuba	Narrow	Low

The cassava common and cassava X viruses and the Caribbean mosaic, frogskin, and latent agents cause the most economically important viral diseases on cassava in Latin America. All cassava germplasm dispatched as *in vitro* propagated plantlets from CIAT are tested for freedom from these agents. A range of traditional (mechanical and graft transmission) and recently developed (immune electron microscopy (IEM), enzyme-linked immunosorbent assay (ELISA) and double-stranded RNA (dsRNA) analysis virus detection methods are employed in the virus testing program at CIAT. There are potential applications of more advanced techniques, such as monoclonal antibodies (MAbs) and nucleic acid hybridization for diagnosis of viruses in Latin American cassava clones.

Cassava Common Mosaic Virus. CCMV, a plant potexvirus, has a wide geographical distribution occurring most frequently in the subtropical regions of Argentina, Brazil, and Paraguay. The concentration of virus in cassava is usually high, allowing efficient mechanical transmission to several indicator plant species. A high titer polyclonal antiserum is available for use in IEM and ELISA tests. Several antigenically similar but biologically distinct isolates of CCMV have been identified (CIAT, 1986) which cannot be differentiated with polyclonal antiserum. Recently, an antigenically distinct strain of CCMV has been reported from Mexico (Zettler and Elliot, 1986). The production of MAbs against a strain of CCMV would likely permit the selection of MAbs with either broad specificity for detecting all strains or with narrow specificity for individual strain identification. MAbs have been produced against potato virus X, a potexvirus (Torrance *et al.*, 1986), and against African cassava mosaic virus (Thomas *et al.*, 1986). Strain-specific MAbs would enable the rapid identification of CCMV strains for accurate mapping of their geographical distribution and for monitoring spread.

Cassava X Virus. CsXV is also a member of the plant potexvirus group. However, in contrast to CCMV, it occurs in very low concentrations in cassava and is difficult to mechanically transmit. A polyclonal antiserum has been produced against the type strain for use in IEM and ELISA. Often the virus concentration in cassava is

below the detection limits of IEM. Although ELISA is more sensitive, multiple sampling over time is necessary to avoid false negative reactions. There is evidence for at least one antigenically similar strain of CsXV (CIAT, 1986). The advantage of MAbs would be realized in increased ELISA sensitivity by reducing the nonspecific background reaction encountered in some polyclonal sera when used in plate-based and membrane-based ELISA (Martin, 1987). MAbs would also be useful for strain diagnosis.

Frogskin and Caribbean Mosaic Disease Agents. The agents causing these diseases have not been characterized. However, a graft transmission test to a susceptible indicator clone is available for detecting both agents in infected cassava clones. Mechanical transmission to other plant species has not been possible. The isolation and purification of these agents directly from infected cassava has not been successful. Based on similar symptomatology and insect transmission properties, they are both likely members of the plant geminivirus group. However, a seriological reaction was not detected with any of the geminiviruses tested. The application of nucleic acid hybridization techniques may aid in the identification and classification of the viruses involved. Spot hybridization (Robinson *et al.*, 1984), restriction fragment patterns (Haber *et al.*, 1987), and Southern blotting (Coutts and Buck, 1987) have been used in the identification of geminiviruses.

Latent Agents. A range of dsRNA species have been detected in apparently disease-free cassava clones (Gabriel *et al.*, 1987). Normally, dsRNAs in plants are associated with a viral infection. The dsRNA analysis technique has been adapted as a general method for detecting latent, unknown RNA plant viruses in cassava (CIAT, 1986). It may be possible to simplify and increase the sensitivity of the standard dsRNA detection method (Dodds, 1984) through the application of (1) serological techniques, using either polyclonal antiserum (G[illegible]l, 1986) or MAbs (Benhamou *et al.*, 1987), or, (2) nucleic acid hybridization (Jordan and Dodds, 1983).

Germplasm Exchange and Conservation

Transfer of genetic resources from the major centers of diversity to other regions of the world is restricted because viral, fungal, and bacterial pathogens tend to persist in the vegetative material, i.e., stakes, cuttings, tubers, corms, etc. Exhaustive pathogen testing of micropropagated plantlets after meristem-tip culture and therapy treatments, substantially minimizes, or eliminates, the risk of pest and disease dissemination. This approach has been implemented, in the last few years, for the international exchange of cassava,

potatoes, and sweet potatoes. (Roca *et al.*, 1979; Schilde-Rentschler and Roca, 1987; Frison, 1981). Elite clones of these crops are routinely distributed in the form of individual well-rooted plantlets in sterile media and contained in test tubes. In addition, cassava and potatoes are also aseptically shipped in larger numbers in plastic containers, and potatoes in the form of *in vitro*-induced tubers. The two latter techniques have been found to greatly facilitate handling (i.e., micropropagation, potting, field transplant) at the receiving end. *In vitro* culture of cuttings, shoot tips, buds, and sexual embryos has been advocated as a means for field collection of primitive and wild genotypes. This technique can be useful for crops such as cacao, where seeds are unavailable or inappropriate, or for collecting expeditions in isolated areas (Withers, 1988).

Small samples of pathogen-tested clones can be maintained *in vitro* to allow new propagation of disease-free plants to be made at any time; *in vitro* maintenance prevents recontamination with pathogens. Large germplasm collections of vegetatively propagated crops are traditionally maintained in the field; this exposes the material to losses due to pest or disease attack, climatic changes, and soil problems. Modifications of micropropagation techniques provide methods for germplasm conservation *in vitro* which can be valuable adjuncts to traditional procedures. Two types of *in vitro* gene banks have been proposed (Withers and Williams, 1986): (a) *in vitro* active gene bank (IVAG) where cultures are maintained under slow growth; and, (b) *in vitro* base gene bank (IVBG) where cultures are cryopreserved. To minimize chances of somaclonal variation, cultures derived from preexisting meristems, e.g., shoots, rooted shoots, plantlets, or embryos, are preferred for genetic conservation (Scowcroft, 1984). The IVAG constitutes a working collection; several of these exist for vegetatively propagated crops; two major ones are the cassava and potato *in vitro* collections at CIAT and CIP, respectively. Techniques for these two crops allows a 1.5-2.0 year subculture interval under reduced temperature and illumination, and modified culture media. However, it is necessary to develop monitoring criteria for genotype stability, culture viability, and other logistical aspects involved in running an IVAG; this work has been initiated at CIAT on a pilot basis, using cassava as a model, in an IBPGR-CIAT collaborative project (Chavez *et al.*, 1988). Although there is not an IVBG in operation, this approach theoretically should enable full maintenance of germplasm genotypic stability. Organized structures like buds, shoot tips, and embryos are also preferred for this technique in order to facilitate retrieval of plants from storage. Generally, liquid nitrogen (–196°C) is used for maintenance of cultures; procedures for hardy fruit tree buds, e.g., apple, pear, have been developed as well as for other

herbaceous species like carrots and strawberries; research into cryopreservation of root tuber crops such as cassava and potatoes is underway (Withers, 1988).

Commercial Micropropagation

Although methodologies exist for tissue culture propagation of nearly 1,000 plant species, only the herbaceous and woody ornamentals, some woody perennials, and a few plantation crops have been micropropagated commercially. Current micropropagation through enhanced axillary branching (Fig. 1) is very labor-intensive; around 40% of the process is used in manual operations (Walker, 1986). Suggestions have been made to develop somatic embryogenesis in continuous liquid culture and embryo encapsulation techniques in order to greatly increase micropropagation efficiency.

There are approximately 150 laboratories dedicated to commercial micropropagation; the majority are located in North America and Europe; some 25 exist in Australia and New Zealand (George and Sharrington, 1984) and a few in Southeast Asia and Latin America (Roca *et al.*, 1988). While the great majority are dedicated to ornamentals, and a few woody fruits and forest trees, commercial production of early generation disease-free potatoes is the only root/tuber crop included. A recent survey with 107 institutions in 23 countries of Latin America and the Caribbean revealed that micropropagation is overwhelmingly the most important research activity, and applications to root/tuber crops are also the most important in public institutions of the region. However, only 10 laboratories are dedicated to commercial micropropagation of ornamentals, industrial crops, temperate fruits, and one root/tuber crop. Links between universities, national agricultural research programs, and the private sector are notoriously weak in the region. For the next five years it is likely that tissue culture will remain as the most important activity in Latin America, followed by molecular and biochemical research (Roca *et al.*, 1988).

Biotechnology Research at CIAT

Tissue culture research at CIAT began 10 years ago, and in 1985 CIAT established the Biotechnology Research Unit (BRU) to function as a link between advanced research institutions, CIAT commodity programs, and national programs, for the application of biotechnology information and tools. The areas of research pursued are: (a) eliminating major constraints to improving production and utilization of CIAT commodities; (b) traditional approaches have shown limited promise; and there is reason to believe that cellular

and molecular biology research and tools can aid in solving the problem; and (c) where widespread application of the technology can be expected.

Applications of Available Techniques. Currently, routine applications of biotechnology to CIAT commodities include tissue culture and biochemical techniques (Table 1). Germplasm management-related constraints were the first to be tackled in cassava. Thus, micropropagation techniques for virus elimination (Roca, 1985), and virus indexing have been successfully applied for viral diseases known in the Americas. CIAT *in vitro* active gene bank (IVAG) now comprises over 3,500 clones, and over 600 elite clones and germplasm accessions have been distributed *in vitro* to national programs in Latin America and Southeast Asia; similarly, nearly 2,000 cassava cultigens have been introduced to CIAT from 12 countries using *in vitro* techniques. To facilitate *in vitro* germplasm exchange, training of national program personnel has been carried out for 15 countries. Trials with micropropagated disease-free clones have resulted in yield gains of up to 100% in many local cassava cultivars. For example, from a few elite cassava clones sent from CIAT, *in vitro* massive multiplication has been carried out at the South China Institute of Botany. In 15 on-farm trails in Guangdong province, average 80% yield increase over local varieties has been obtained with micropropagated clones of a CIAT-improved genotype. Planting of 30,000 hectares in the Guangdong province has been projected for 1989. (K. Chun-Yen, personal communication). Isoenzyme electrophoretic techniques (Hussain *et al.*, 1987) have enabled researchers to identify duplicates among cassava germplasm accessions. Clustering of duplicated accessions from the collection will facilitate germplasm management.

Table 1. Current Applications of Available New Techniques to CIAT Commodities (April, 1988).

Constraints	Techniques
CASSAVA	
1. Restricted international exchange of clones	1. Disease elimination
2. Problems of germplasm field conservation	2. Slow growth *in vitro*
3. Yield decline of local cvs.	3. Micropropagation
4. Redundancy in germplasm accessions	4. Isoenzyme electrophoresis

Table 1. Current Applications of Available New Techniques to CIAT Commodities (April, 1988).—Continued

Constraints	Techniques
RICE	
1. Long breeding cycle ecosystems	1. Anther culture breeding
COMMON BEANS	
1. Dispersal/evolution of germplasm in Africa	1. Phaseolin electrophoretic markers
TROPICAL PASTURES	
1. Restricted exchange of *Brachiaria*	1. Micropropagation

Based on the work by Gepts and Bliss (1986), electrophoretic patterns of common bean phaseolin have been used to track the evolution and dispersal of *P. vulgaris* germplasm between Latin America and Africa. Phaseolin electrophoretic types are correlated with the major common bean domestication centers in the Americas.

Rice anther culture techniques have been improved at CIAT (CIAT, 1984) and used by CIAT rice program breeders to produce thousands of doubled haploid lines to address breeding problems in the Southern Cone and the upland, savanna, soils of Latin America. This project is part of the Rockefeller Foundation rice biotechnology network. In those ecosystems, only one generation can be grown per year, extending the breeding process to 12-15 years. Major constraints in Southern Cone rice are cold and poor grain quality. In 1987, cold-tolerant and high-quality grain rice lines were selected out of 800 doubled haploids produced three years before for this target ecosystem (CIAT, 1987).

Methodology Development Research. Methodological research aims at improving current routine procedures and developing new approaches to selected constraints in CIAT commodities (Table 2). Under a special collaborative project with IBPGR, monitoring techniques (morphological, cytological, biochemical) are being developed for assessment of genotypic stability and culture viability at various stages of *in vitro* storage of cassava germplasm (Chavez *et al.*, 1988).

Table 2. Methodological Development in Biotechnology at CIAT (April, 1988).

Constraint	Methodological Approach
CASSAVA	
1. Monitoring needs in *in vitro* conservation	1. Pilot IVAG*
2. Regeneration problems	2. Somatic embryogenesis
3. Cyanogenesis	3. Haploid induction/ mutagenesis
COMMON BEANS	
1. Regeneration recalcitrancy	1. Tissue culture of wild *P. vulgaris*
2. Gene pool incompatibility	2. Isoenzyme Electrophoresis**
TROPICAL PASTURES	
1. *S. guianensis* improvement	1. Somaclonal variation Protoplast fusion
2. Slow identification *Brachiaria recombinants*	2. Isoenzyme Electrophoresis**

* Special project with IBPGR
**Special projects with University of Manitoba, Canada

Research to regenerate cassava plants from somatic and gametophytic tissues is underway at the BRU. While a considerable number of cassava plants regenerated through somatic embryogenesis have been moved to the field for evaluation of agronomic performance (Szabados *et al.*, 1987; CIAT, 1987), the haploid induction project has just began. Since acyanogenesis seems under recessive genetic control, microspore-derived cassava plants should display variability for this trait.

Research on common beans has resulted in the regeneration of plants on callus derived from embryogenic axis of several wild relatives of *P. vulgaris* (CIAT, 1988). This research complements collaborative efforts at Colorado State University with *P. acutifolius*, and in Viterbo, Italy with *P. vulgaris* (Table 3). On the other hand, isoenzyme electrophoretic analysis is being developed to study the genetic relationships between germplasm accessions of *P. vulgaris* from the gene pools of Mesoamerica and Southern Andes, where restriction to recombination exists.

Table 3. CIAT Collaborative Research Projects in Biotechnology (April, 1988)

Project	Institution
CASSAVA	
1. Cryopreservation of meristems	IBPGR/PBI, Canada
2. rDNA for gene transfer	LSU, Baton Rouge
COMMON BEANS	
1. Molecular markers for geographic distribution of variability	Univ. California, Davis
2. RFLP's markers for BCBB	Univ. Florida, Gainesville
3. Regeneration of *P. acutifolius*	TCCP, CSU, Fort Collins
4. Regeneration of *P. vulgaris*	Inst. Biol. Agr., Viterbo, Italy
5. rDNA for gene transfer	Inst. Ortic., Montanaso Italy

In the tropical forage legume *Stylosanthes guianensis*, a wide range of heritable tissue culture-generated variability (e.g., chromosome doubling, leaf area, growth habit, seed production, anthracnose tolerance, etc.) has been identified in the progeny of plants generated from callus cultures (CIAT, 1987). Plants have been regenerated from *S. guianensis* + *S. capitata* protoplast fusion products (Szabados and Roca, 1986). Isoenzyme electrophoretic analyses have shown the combination of parental bands at two loci in several plants. The objective of this research is to transfer *S. capitata* traits to *S. guianensis*.

CIAT Collaborative Projects in Biotechnology. A total of seven collaborative projects have been established in the last two years with advanced research institutions of the USA, Canada, and Europe to address key research constraints in cassava and common beans (Table 3).

Early success in cassava cryopreservation at the Plant Biotechnology Institute, Canada (Kartha *et al.*, 1982) has stimulated efforts to develop the technique further. An IBPGR postdoctoral fellow has begun research in this area in Canada and will be transferring the technology to CIAT in the near future. Research at Louisiana State University to develop *Agrobacterium*-mediated gene transfer techniques began last year; evidence of cassava tissue transformation has been shown through a kanamycin resistance gene (J. Jaynes, personal communication).

In common beans, collaborative projects include both cellular and biochemical/molecular research. At the University of Florida, development of DNA-RFLP's as molecular genetic markers to tag bean common bacterial blight (BCBB) resistance has been initiated this year. Twenty-five cDNA and genomic clones have been hybridized to restriction digests of cv. "Calima" and the BCBB line "XR-235-1." Out of these, 11 have revealed polymorphisms between the two lines (Vallejo and Chase, 1988). BCBB was selected because of the difficulties encountered in progeny screening and backcrossing. Collaborative projects in two Italian institutions aim at developing the components for an *in vitro* gene manipulation strategy in beans, i.e., plant regeneration and gene transfer techniques (Table 3).

Constraints Requiring Generation of New Information and Techniques. CIAT is in the process of identifying constraint priorities in *Manihot* and *Phaseolus* that require networking of stronger collaborative research to generate basic information and techniques for the application of biotechnology. Projects that are already underway (Tables 2 and 3) will be a natural part of the larger network. Among the major constraints identified at this time for collaborative research are: cyanide (HCN) toxicity, postharvest physiological root deterioration, propagation-related constraints, photosynthetic capacity under stress, nutritional quality, viral diseases, and insect pests. Some of these constraints may be approached with some of the emerging biotechnologies, e.g., virus resistance through incorporation of virus capsid protein genes or insect resistance by the B.T.i. gene. Others, however, would require previous biochemical/genetical research to identify possible targets for application of biotechnological tools.

Work is in progress to prioritize constraints on cassava production. A similar exercise will then be followed for common bean.

References

Ammirato, P.V. 1983. Embryogenesis, *in* Handbook of Plant Cell Culture, Vol I (Evans, D.A.; Sharp, W. R.; Ammirato, P.V.; and Yamada, Y.; eds.), Macmillan Publishing Co., New York, pp. 82-123.

Benhamou, N.; Parent, J.G.; Garzon, S.; Asselin, A.; Ovellette, G.B. and Joly, J.R. 1987. Use of monoclonal antibody against poly (I): poly (c) for detecting mycoviruses and potential applications to potato spindle tuber viroid and animal reoviruses. Canadian Journal of Plant Pathology 9: 106-114.

Bondary, K.R.; and Styer, Ch. 1985. Scaling up plant cloning: Spin filter bioreactor process technology, Gen. 5: 22 and 28.

CIAT. 1984. El cultivo de anteras: un camino más corto para obtener variedades mejoradas, *in* Arroz en las Americas 5: 1-5, CIAT, Cali, Colombia.

_____. 1986. Cassava Program Ann. Report, 1985. CIAT, Cali, Colombia.

_____. 1987a. CIAT Report 1987, Cali, Colombia, pp. 20-22.

_____. 1987b. Tropical pastures, *in* Biotechnoiogy Research Unit Ann. Report 1986, CIAT, Cali, Colombia.

_____. 1988a. Biotechnology research, *in* Bean Program Ann. Report 1987, CIAT, Cali, Colombia.

_____. 1988b. Cassava Program Ann. Report, 1987. CIAT, Cali, Colombia.

Chavez, R.; Roca, W.M.; and Williams, J.T. 1988. IBPGR-CIAT collaborative projects on a pilot *in vitro* active gene bank, FAO/IBPGR Plant Genetic Resources Newsletter 71: 11-13.

Coutts, R.H.A. and Buck, K.W. 1987. Cassava latent virus specific DNAs in mosaic diseased cassava of Nigerian origin. Neth. J. Pl. Path. 93:241-244.

Dodss, J.A.; Morris, T.J.; Jordan, R.L. 1984. Plant viral double-stranded RNA. Ann. Rev. Phytopathol. 22: 151-168.

Frison, E.A. 1981. Recommendations for transporting and handling tissue culture material, sweet potato and cassava, Manual Series No. 6, IITA Ibadan, p. 10.

Gabriel, C.J. 1986. Detection of double-stranded RNA by immunoblot electrophoresis. Journal of Virological Methods. 13: 279-283.

Gabriel, C.J.; Walsh, R.; and Nolt, B.L. 1987. Evidence for a latent virus like agent in cassava. Phytopathology 77: 92-95.

George, E.F.; and Sherrington, P.D. 1984. Plant propagation by tissue culture. Handbook and Directory of Commercial Laboratories, Exegetics Limited, U.K.; p. 709.

Gepts, P. and Bliss, F. 1986. Phaseolin variability among wild and cultivated common beans (*Phaseolus vulgaris*) from Colombia, Econ. Botany 40: 469-478.

Haber, S.; Polston, J.E.; Bird, J. 1987. Use of DNA to diagnose plant diseases caused by single-strand DNA plant viruses. Can. Jour. Plant Pathol. 9: 156-161.

Hussain, A.; Bushuk, W.; Ramirez, H.; and Roca, W.M. 1987. Identification of cassava (*Manihot esculenta* Crantz) cultivars by electrophoretic patterns of esterase izoenzymes. Seed. Sci. and Technolog. 15: 19-22.

Jordan, R.L. and Dodds, A. 1983. Hybridization of 5-End-Labelled RNA to plant viral RNA in agarose and acrylamide gels. Plant Molecular Biology Reporter. 1: 31-37.

Kartha, K.K.; Leung, N.L.; Mroginski, L.A. 1982. *In vitro* growth responses and plant regeneration of cryopreserved meristems of cassava (*Manihot esculenta* Crantz). A Planzenphysiol. Bd. 107: 133-140.

Lozano, J.C.; Pineda, B.; and Jayasinghe, U. 1983. Effect of cutting quality on cassava (*Manihot esculenta* Crantz) performance *in* Proc. 6th Intern. Trop. Root Crops Symp., Lima, Peru.

Martin, R.R. 1987. The use of monoclonal antibodies for virus detection. Can. Jour. Plant Pathol. 9: 177-181.

Robinson, D.J.; Harrison, B.D.; Sequeira, J.C. and Duncan, G.H. 1984. Detection of strains of African cassava mosaic virus by nucleic acid hybridization and some effects of temperature on their multiplication. Ann. Appl. Biol. 105: 483-493.

Roca, W.M.; Bryan, J.E.; and Roca, M.R. 1979. Tissue culture for the international transfer of potato genetic resources. Am. Potato J. 56: 1-10.

Roca, W.M.; Amezquita, M.C.; and Villalobos, V.M. 1987. Directorio de Biotecnología Agrícola por especies y tecnologías en America Latina y el Caribe. Encuesta, 1986. CIAT, Cali, Colombia, p. 86.

Roca, W.M.; Amezquita, M.C.; and Villalobos, V.M. 1988. Estado actual y perspectivas de la Biotecnología Agrícola en America Latina y el Caribe. Encuesta 1986, *in* Proc. Intern. Seminar. Temas Prioritarios Mecanismos de Coop. en Invest. Agrop. en America Latina y el Caribe, CIAT, Cali, Colombia, pp. 187-212.

Reeves, J.W. 1986. New rice varieties in half the time, Span 29: 32-33.

Scowcroft W.R. 1984. Genetic variability in tissue culture: Impact on germplasm conservation and utilization. AGP/IBPGR/84/152, IBPGR, Rome.

Schilde-Rentscheler, L.; and Roca, W.M. 1987. Tissue culture for the international exchange of potato and cassava germplasm, *in* Biotechnology in Agriculture and Forestry, Vol. 3. (Bajaj, Y P.E. ed.) Springer-Verlag, Berlin, pp. 453-465.

Schilde-Rentscheler, L., and Roca, W.M. 1986. Virus elimination in potato and cassava, *in* Global workshop on root and tuber crops propagation, Proc. Workshop, CIAT, Cali, Colombia, pp. 89-95.

Szabados, L.; and Roca, W.M. 1986. Regeneration of isolated mesophyll and cell suspension protoplasts to plants in *Stylosanthes quianensis*. A tropical forage legume. Plant Cell Reports 5: 174-177.

Szabados, L.; Hoyos, R.; and Roca, W.M. 1987. *In vitro* somatic embryogenesis and plant regeneration of cassava. Plant Cell Reports 6: 248-251.

Torrance, L.; Larkins, A.P. and Butcher, G.W. 1986. Characterization of monoclonal antibodies against potato virus X and comparison of serotypes with resistance groups. J. Gen. Vir. 67, 57-67.

Thomas, J.E.; Massalski, P.R. and Harrison, B.D. 1986. Production of monoclonal antibodies to Africa cassava mosaic virus and differences in their reactivities with other whitefly-transmitted geminiviruses. J. Gen. Virol. 67: 2739-2748.

Walker, K.A. 1986. Automation of *in vitro* plant propagation: Has its time finally arrived? Gen. 6: 52.

Withers, L.; and Williams, J.T. 1986. *In vitro* conservation, IBPGR, FAO, Rome, p. 21.

Withers, L. 1987. Long-term preservation of plant cells, tissues and organs, *in* Oxford Surveys of Plant Molecular and Cell Biology, 4: 221-272.

Zettler, F.W. and Elliot, M.S. 1986. An antigenically distinct strain of cassava common mosaic virus *infecting (*Nidoscolus aconitifolius*). Phytopathology 76: 632-638.

Breeding for Rice Varieties Tolerant to Adverse Conditions Through Tissue Culture at IRRI

F.J. Zapata, Tissue Culture Specialist
and
L.B. Torrizo, Senior Research Assistant
International Rice Research Institute
Manila, Philippines

The fast rate of world population increase is constantly imposing pressure on the agricultural sector to produce enough food (both in quantity and quality). This problem is being attacked on two fronts—to increase productivity in the available arable land, and to utilize the currently idle lands which mainly are comprised by those exposed to suboptimal conditions for crops' growth. Significant increases in crop productivity have progressively become difficult to obtain since the gap between actual and potential yields has increasingly become narrower due to better crop management and higher-yielding varieties. On the other hand, considerable yield increases could be gained in the second alternative due to the vast areas of land subjected to adverse conditions such as salinity (Table 1), drought (Table 2), and other stresses.

Table 1. Distribution of Saline Lands (Ponnamperuma, F.N., 1984).

	Area (million ha)		
Region	Strongly Saline	Moderately Saline	Total
Africa	16.5	37.0	53.5
Australasia	16.6	0.79	17.4
Mexico and Central America	0.24	1.72	1.96
North America	0	6.2	6.2
South America	10.5	58.9	69.4
North and Central Asia	22.5	69.2	91.7
South Asia	47.2	36.1	83.3
Southeast Asia	0	20.0	20.0
Total	113.5	230.0	343.5

Table 2. Estimated Upland Rice Area in the World (Tran Van Dat, 1986).

Region	Area (thousand ha)	Production (thousand t)	Yield (t/ha)
Africa	2047	1023	0.50
Asia	11593	11593	1.00
Latin America	6724	8820	1.10
World	20364	21436	1.05

Rice ranks first in importance as a food crop since it provides more calories than any cereal on a per hectare basis, although it is second to wheat in terms of area harvested (De Datta, 1981). Breeding for rice varieties takes several years since it takes several generations of selfing until homozygosity is reached. Although wild rices (Table 3) could be useful in improving the resistance to pests, diseases, and adverse environment of the cultivated species, crossability barriers often have to be overcome. Since the objective is to breed desirable genotypes best suited to the adverse growth conditions at the shortest possible time, and being aware of the limitations of conventional breeding, some areas in crop improvement have been identified where biotechnology, which includes tissue culture, could be valuable (Khush and Virmani, 1985).

Table 3. Wild Species of *Oryza* with Useful Traits (Swaminathan, 1986).

Wild Species	Useful Trait
O. perennis	Tolerance to stagnant flooding and acid sulfate soils
O. navara	Resistance to grassy stunt virus and blast
O. officinalis	Resistance to BPH, WMBPH and GLH
O. australiensis	Resistance to BPH and drought
O. barthii	Resistance to bacterial leaf blight
O. longistaminata	Floral characters for out pollination
O. coarctata	Tolerance to salinity

The tissue culture methods employed at the International Rice Research Institute include anther, isolated pollen, somatic cell, and protoplast cultures. In the future, protoplast fusion and DNA transfer techniques will be incorporated. These methods are utilized

at IRRI as they are applicable in the breeding of varieties adapted to adverse conditions through: (1) immediate fixation of homozygosity for faster production of stable lines, (2) generation of somaclonal/gametoclonal variants, (3) somatic hybridization, and in the future, (4) gene transfer techniques. As these innovative breeding techniques are relatively new technologies, most of our research is still basic in nature, which ultimately will be of practical value in the pursuance of our research objectives.

Immediate Fixation of Homozygosity

Among the different methods of haploidy, anther and isolated pollen cultures are the most practical as pollen grains come in large numbers. The advantages of using haploids in breeding have been discussed by Snape (1982). Since this technology requires plant production in large numbers, basic research on increasing callus and plant regeneration efficiencies is constantly being undertaken.

Analysis of Genetic Control on Callus Induction and Plant Regeneration Using Diallel Crosses

Japonicas are generally responsive, while Indicas are considered recalcitrant to anther culture. Although *in vitro* culture conditions could be made optimal to effect maximum response from the anthers, incorporation of responsiveness into the Indicas through the genes may have more-lasting results. Thus, inheritance of callus induction and plant regeneration was studied using 16 genotypes from a 4-parent diallel set. Both callus induction and green plant regeneration were indicated to be highly recessive characters with large amounts of genetic variance due to additive gene effects. Partial dominance and nuclear control of these characters were also indicated (Quimio and Zapata, 1988a and b). These results suggest the possibility of transferring the high culturability of the Japonicas into Indicas by proper choice of parents.

Other Factors Affecting Androgenesis

Aside from the influence of genetic factors on androgenesis, the effect of culture conditions, media and media components, physical treatments and physiological state of the donor plants is also being studied. Physical treatments such as irradiation (Zapata et al., 1986) and heat treatment (Zapata and Torrizo, 1986), for example, have increased callus induction and plant regeneration. Irradiation dosage of 20 krad to the seeds for panicle collection resulted in 26% green plant regeneration while there was no regeneration in

the control. On the other hand, using IR42 as test variety, heat treatment of panicles at 35° for 15 minutes prior to anther culture increased callus production by as much as 100% relative to the control.

Chemical factors such as sucrose, abscisic acid (ABA), and conditioned medium also significantly affect culturability. Increasing concentrations of sucrose up to 12% resulted in increased callus induction. Likewise, plant regeneration (both green and albino) was enhanced (Mercy and Zapata, 1986). Addition of abscisic acid into callus induction and plant regeneration media enhanced anther culture efficiencies. ABA at a concentration of 2.5 mg/l was optimum for callus induction. For semisolid and liquid media, the optimum duration of exposure was for 2 and 3 days, respectively (IRRI, 1987). Likewise, stressing anther-derived calli in ABA-containing medium before transferring them to regeneration medium without ABA significantly increased green plant regeneration (Torrizo and Zapata, 1986). Earlier findings by other researchers showed that ABA given for short periods increased pollen embryogenesis in tobacco anthers (Imamura and Harada, 1980) and stimulated shoot bud and plantlet formation in rice somatic callus cultures (Inoue and Maeda, 1981).

Likewise, culturing anthers on conditioned medium not only increased callus induction but also enhanced green plant regeneration in some varieties (Zapata *et al.*, 1985).

Isolated pollen culture. As in anther culture, this technique likewise provides a method of recovering homozygous plants from heterozygous materials in a single generation. Compared to anther culture, however, the efficiency is still low. It has an advantage over anther culture, however, in that possible inhibitory effects of the anther wall on microspore development are eliminated. Furthermore, microspores can be manipulated in the same manner as single cells and are thus appropriate for selection of variants. Pollen grains are also natural cellular "vectors" and have a potential use in genetic transformations. Foreign DNA can be introduced into the developing male gametophyte and transported to the embryo sac as a part of regular fertilization process.

A routine procedure has been established in obtaining callus induction and green plant regeneration in Taipei 309. Preculture of anthers before pollen isolation and addition of amino acids to callus induction medium greatly increased callus induction and subsequent plant regeneration (Cho and Zapata, 1988). Table 4 shows varieties being used and response observed. We are optimistic that positive results in Indicas will soon follow. Getting considerably

higher efficiency using this technique will definitely improve the screening and selection system at cellular level for recombinants tolerant to various stresses.

Table 4. Response of Various Rice Varieties to Isolated Pollen Culture.

Varieties	Response
Japonicas	
Taipei 309	Plant regeneration
RAC 3	Plant regeneration
*Indicas**	
IR36	Multicellular pollen grains
IR42	Callusing
IR43	Callusing
Pokkali	Multicellular pollen grains
IR54754A	Callusing
IR54754B	Callusing

*Experiment in progress.

Development of lines for adverse conditions. One of the main activities in our laboratory is the use of anther culture in the fixation of homozygosity from F1 crosses bred for different adverse conditions. These projects are in collaboration with different institutions and IRRI plant breeders. Tolerance to salinity, drought, cold, blast, and hoja blanca are being worked on. Summary of the data on these projects are shown in Table 5.

Table 5. Green Plant Regeneration in Various Collaborative Projects on the Production of Homozygous Lines Tolerant to Adverse Conditions through Anther Culture.

Adverse Condition Bred For	Collaborating Country	Crosses Plated for Anther Culture (No.)	Green Plants Produced (No.)	Lines Tested (No.)	Lines Selected (No.)
Cold tolerance	Korea	5[illegible]	1[illegible],[illegible]15	1367	13*
Resistance to Blast/Hoja blanca	Peru	25	781	273	3
Upland conditions	In-house (M. Arradeau)	107	1,988	419	25
Salt tolerance	In-house (D. Senadhira)	101	1,622	65	13

*One line nominated as Suweon 357.

a. **Cold tolerance.** Screening of anther culture-derived materials is done in Suweon, Korea by the Rural Development Administration and in the International Rice Cold Tolerance Nursery (IRCTN) by the International Rice Testing Program, IRRI. One line has been nominated as Suweon 357 from among 13 lines selected for replicated yield trials (Table 6). This line is earlier-maturing, has shorter plant height, and most important, has resistance to blast and leaf blight among its advantages over the anther culture-derived variety Hwaseongbyeo and the check Daelwanbyeo. Suweon 357 will be retested in replicated yield trials and tested in the regional adaptability test nursery in 1988. Thus, this possibly cold-tolerant and blast-resistant variety developed through anther culture took around 3 years, as against 10 or more years it would take by conventional breeding in countries like Korea where there is only one cropping season per year. Aside from the 13 lines selected earlier, another 31 lines were selected in 1987 and these will be tested in the yield trial nursery this year.

On the other hand, one line (from Taipei 309/Tatsumi mochi) of three lines tested was comparable to the resistant check, Stejaree 45. This screening was conducted in 15 locations composing IRCTN.

b. **Salinity tolerance.** Three anther culture-derived lines were compared to the resistant check, Pokkali, at different levels of electrical conductivity (Table 7). Two lines, AC6530 and AC6526, were comparable to the check in some aspects such as percentage survival, relative leaf area, and relative leaf weight. In some cases such as in relative sheath and root weight, AC6530 and 6526 were even better than Pokkali at EC = 5. Anther culture lines from crosses bred for salt tolerance were tested under field conditions, and 13 out of 65 were selected.

IRRI breeders found that some F1 anther culture derivatives of crosses designed for improving salt-tolerant traditional cultivars exhibited good agronomic traits as well as salt tolerance level comparable to the donor parent, a process that would have taken more than a decade in conventional breeding (Senadhira, 1988). Whether the vigor of the anther culture lines was due to the stress induced by in vitro culture or the possible selection of gametes will need further investigations. The fact remains though, that some steps in the breeding process can be replaced or improved by the tissue culture methods.

Table 6. Replicated Yield Trials of Selected Cold-tolerant Lines (Suweon, 1987).

Genotype	Days to Head.	Height (cm)	Plant Panicles (no.)	No. of Grains /pani.	1000 Grain wt (gr)	Filled Grain (%)	Grain Yield (t/ha)	WC/WB (0-9)	Blast (0-9)	BLB	Remarks
SR12197-T7	107	94	17	79	22.1	74.3	5.3	wx	2	R	
SR12197-T8	107	95	16	85	22.4	69.7	5.8	wx	2	R	Suweon 357
SR12212-T2	127	116	14	95	22.8	80.7	5.1	0/1	8	S	
SR12212-T4	126	106	14	79	23.4	84.7	5.1	1/0	8	S	
Hwaseongbyeo	117	104	14	97	23.5	87.3	6.1	0/0	9	S	
SR11452-T231	95	71	15	57	26.1	62.5	3.2	wx	0	—	
SR11452-T232	95	76	19	80	24.8	65.0	3.3	wx	0	—	
SR11452-T233	97	72	15	64	26.0	78.0	3.0	wx	0	—	
SR11452-T234	97	73	15	55	27.0	83.0	3.4	0/0	0	—	
SR12200-T5	124	104	16	83	22.2	78.0	5.6	0/0	9	—	
Hwaseongbyeo	119	103	15	94	21.9	77.5	6.1	0/0	9	—	
SR11452-T305	97	92	11	57	—	50.7	2.9	—	3	S	
SR11453-T107	110	108	21	35	—	52.3	4.9	—	3	S	
SR11453-T277	107	97	15	42	—	41.1	4.7	—	3	S	
Daelwanbyeo	112	105	16	65	--	50.2	6.1	—	3	S	

Notes: Genotypes 1-11
Seeding: April 15
Transplanting: May 25
Fertilizers: N-P_2O_5-K_2O = 120-90-110
Spacing: 30 x 15 cm (3 plants/hill)

Genotype 12-16
Seeding: April 1
Transplanting: May 12
Fertilizers: N-P_2O_5-K_2O = 120-70-80
Spacing: 30 x 15 cm (3 plants/hill)

Table 7. Response of Anther Culture-derived Rice to Salinity Based on Relative Growth of Various Parameters.

Electrical Conductivity	Variety/Line[1]			
	Pokkali	AC6530	AC6526	AC6533
		Survival (%)		
0	100	100	100	100
5	100	100	100	100
10	100	99	94	100
		Relative Leaf Area		
0	1.0	1.0	1.0	1.0
5	0.84	0.80	0.87	0.65
10	0.66	0.45	0.41	0.44
		Relative Leaf Weight		
0	1.0	1.0	1.0	1.0
5	0.95	0.92	0.94	0.74
10	0.82	0.65	0.62	0.54
		Relative Sheath Weight		
0	1.0	1.0	1.0	1.0
5	0.94	1.07	1.02	0.78
10	0.94	0.69	0.67	0.50
		Relative Root Weight		
0	1.0	1.0	1.0	1.0
5	1.05	1.06	1.19	0.81
10	1.16	0.78	0.88	0.69
		Relative Total Weight		
0	1.0	1.0	1.0	1.0
5	0.96	0.98	1.0	0.75
10	0.90	0.68	0.67	0.54

[1]Pokkali—resistant check; AC6530 and 6526 = IR5657-33-2/IR4630-222-5-1-3; AC6533 = IR4630-22-2-5-1-3/Pokkali.

c. **Other studies on tolerance to various stresses.** In-house collaboration on the development of lines tolerant to upland conditions resulted in the selection of 25 out of 419 lines tested (Table 5). Likewise, 3 of 273 lines from crosses bred for resistance to blast and hoja blanca were selected.

In a study conducted by CIAT comparing one of our promising anther culture lines (AC800-1) with their checks under favored upland conditions, AC800-1 flowered earlier than the two checks and was more resistant to Bl, NBl and BS than one or both check varieties (Table 8).

Table 8. Performance of Anther Culture-derived Line (AC800-1) and Two Checks under Favored Upland Conditions (CIAT, 1987).

Lines	Days to Flower	Diseases[1]			
		Bl	NBl	LSc	BS
AC800-1	88	2	4	4	3
Checks					
Oryzica 1	99	4	5	3	8
Cica 8	109	5	5	3	2

[1]Bl = Leaf blast; NBl = Neck and node blast; LSc = Leaf scald; BS = Brown spot.

BS evaluations were conducted in Alanje, Panama; the rest in Villavicencio, Colombia.

In an earlier study at IRRI, the sister lines AC800 and 800-1 from the cross IR48/Suweon 290 consistently yielded higher than the parents in replicated yield trials for 3 seasons (Table 9). Further investigation showed higher harvest indices of the said anther culture lines. Other distinguishing features observed included earlier maturity, higher tillering, erectness and shorter plant height, and increased resistance to rice tungro virus of the anther culture lines compared to the parents.

Table 9. Agronomic Data of Selected Anther Culture-derived Lines and Parents (IRRI, 1985-1986).

Designation	Grain Yield			Harvest Index	
	1985WS	1986DS	1986WS	1986DS	1986WS
AC800	5.43 AB*	5.65 ABC	4.65 AB	0.53 A	0.50 A
AC800-1	5.87 A	6.25 A	5.15 A	0.53 A	0.51 A
IR48	4.97 BC	5.03 B-E	3.61 DEF	0.38 C	0.32 C
Suweon 290	2.92 D	5.60 A-D	4.50 BC	0.44 B	0.37 B

*In a column, numbers followed by the same letter are not significantly different from each other.

Generation of Somoclonal and Gametoclonal Variants

Somaclonal or gametoclonal variation, which is the term applied to enhanced genetic variability in plants regenerated from tissue culture, could also be in some cases utilized for the production of lines tolerant to various stresses (Scowcroft *et al.*, 1985, 1987; Evans *et al.*, 1984). Various possible mechanisms for somaclonal variation have been indicated (Scowcroft and Larkin, 1982).

Somaclonal and gametoclonal variations in rice have been reported (Zhao *et al.*, 1985; Oono, 1985). In some cases, positive variations such as increase in productive tiller, fertility, and decreased plant height were produced.

Reports on somaclones adapted to adverse environmental conditions include improvement of salt-tolerant lines by cellular selection (Woo *et al.*, 1984). Sixty percent of the lines regenerated were reported. In our studies on stressing cells with NaCl at 15 g NaCl/liter, we were able to regenerate lines after 32 months (Zapata and Abrigo, 1986). The regenerated plants, however did not survive when grown in culture solution containing 5-15 g NaCl/liter and those kept in solution without NaCl showed 100% sterility. This exemplifies a case where the regenerated plants did not carry the NaCl tolerance trait present in the calli.

Variants that are more resistant to diseases could be possible to obtain through tissue culture. Two somatic cell culture lines from IR43, which have been selected from 125 lines in observational yield trials, were subjected to advanced yield testing and screened for disease/insect reactions (Table 10). Aside from the statistically significant increase in yield in one somatic culture line (IR453SC3-8), it was also more resistant to rice tungro virus. Physiological studies will be conducted to pinpoint the factors that account for the increased yield of this somaclonal variant.

Table 10. Yield and Reaction to Diseases/Pest of Somatic Culture Lines of IR43 and Parent (IRRI, 1987).

Designation	Yield (t/ha)	RTV (%)	Blast	BPH 1	BPH 2	BPH 3
IR43SC3-8	3.34 A	62.0	5	5	7	5
IR43SC1-7	2.37 B	77.5	5	5	5	5
IR43	2.04 B	79.0	6	7	7	7

Somatic Hybridization

One of the limitations in sexual hybridization is the difficulty in realizing crosses between interspecific and intergeneric species. Since wild species possess most of the characteristics for resistance to pests and diseases that are lacking in the cultivated genotypes, it is necessary to find other means of hybridization such as protoplast fusion, otherwise known as somatic hybridization. In addition, somatic hybridization allows the expression of cytoplasmic traits from both parents in comparison to sexual hybridization where only the cytoplasmic characteristics from the female parents are transmitted to the offspring.

So far, the only known successful gene transfer through interspecific hybridization is the incorporation of grassy stunt virus resistance from *Oryza nivara* into *Oryza sativa* (Khush *et al.*, 1977; Goodman *et al.*, 1987). The transfer of brown planthopper resistance from *Oryza officinalis* into cultivated species was done using embryo culture techniques (Jena and Khush, 1984). The Chinese have likewise reported the screening of plants from the cross *Oryza rufipogon* x *Oryza sativa* for resistance to blight and good grain quality (Swaminathan, 1987).

Before attempting somatic hybridization, two very important characteristics have to be demonstrated, i.e., the ability to form plants through somatic embryogenesis and to regenerate plants from protoplasts. Basic research has been conducted on these aspects in preparation for future work on protoplast fusion.

Plant regeneration through somatic embryogenesis from mature seed and young inflorescence of the wild rice *Oryza perennis* was observed (Wang *et al.*, 1987). The frequency of callus induction was higher from young inflorescences (62%) than from mature seeds (42%) as explant. Green plant regeneration frequency in both explants decreased with increasing duration in culture. The regeneration capacity of the calli from the two types of explant did not differ significantly. However, mature seeds are more practical sources of explants since these can be stored and are more readily available any time of the year.

Formation of embryogenic calli and plant regeneration was likewise observed in somatic cultures of *Echinochloa glabrescens* using young inflorescences and leaf segments as explants (Wang and Zapata, 1987). On the other hand, *Echinochloa glabrescens* is a gramineae which can be adapted for cultivation as a forage and grain crop in arid and semiarid regions, as a single watering suffices from germination to harvest. The callus induction frequency was

91% in young inflorescence and 41% in leaf segments. The green plant regeneration efficiency decreased from the 2nd to 6th passages with young inflorescence as better explant than young leaf segment in terms of plant regeneration.

Although several researchers have demonstrated the ability to regenerate plants from protoplasts isolated from suspension cultures derived from different explants such as basal leaf segments and scutella (Abdullah *et al.*, 1986), anther-derived calli (Toriyama and Hinata, 1985, 1986), embryo callus (Shimamoto *et al.* 1986), immature embryo calli (Fujimura *et al.*, 1986), intact seeds (Coulibaly and Demarly, 1986; Yamada *et al.*, 1986), and root tips (Zimny *et al.*, 1986), no successful protoplast fusion between different rice species has been reported except for the rice-barnyard grass somatic hybrids (*Echinochloa oryzicola*) (Terada *et al.*, 1987).

Protoplast culture at IRRI was initiated in 1987 using the model variety Taipei 309. Plant regeneration has been realized and our present work includes other varieties and wild species. The status of response to protoplast culture of these are shown in Table 11. From the knowledge gained in the basic studies, we hope to include somatic hybridization in our future research work.

Table 11. Response of Various Rice Varieties to Protoplast Culture.

Genotype	Response
Japonicas	
Taipei 309	Green plant regeneration
Taipei 177*	Callusing
RAC3*	Callusing
Indicas	
Kulu	Albino plant regeneration
Tetep*	Division

*Experiment in progress.

Besides the use of protoplasts in somatic hybridization, these are now being utilized in gene transfer techniques (Cocking and Davey, 1987; Goodman *et al.*, 1987). IRRI will also venture on this aspect as a valuable research area.

With the fast developments in tissue culture technology coupled with the identification, isolation, and cloning of genes for resistance/tolerance to various stresses, development of rice and other crops for adverse conditions through innovative methods may be realized sooner than expected.

References

Abdullah, R., E.C. Cocking, and J.A. Thompson. 1986. Efficient plant regeneration from rice protoplasts through somatic embryogenesis. Biotechnology 4:1087-1090.

Cho, M.S. and F.J. Zapata. 1988. Isolated pollen culture of rice (*Oryza sativa* cv. Taipei 309). Submitted for publication.

Cocking, E.C. and M.R. Davey. 1987. Gene transfer in rice. Science 236:1259-1262.

Coulibaly, M.Y. and Y. Demarly. 1986. Regeneration of plantlets from protoplasts of rice, *Oryza sativa* L. Z. Pflanzenzuchtg. 96:79-81.

De Datta, S.K. 1981. Principles and practices of rice production. John Wiley and Sons, Inc., Singapore. 618 pp.

Evans, D.A., W.R. Sharp, and H.P. Medina-Filho. 1984. Somaclonal and gametoclonal variation. Amer. J. Bot. 71:759-774.

Fujimura, T., M. Sakurai, H. Akagi, T. Negishi, and A. Hirose. 1986. Regeneration of rice plants from protoplasts (Abstract). In: VI International Congress of Plant Tissue and Cell Culture. 3-8 August, 1986. Univ. of Minnesota, U.S.A. p. 161.

Goodman, R.M., H. Huptli, A. Crossway, and V.C. Knauf. 1987. Gene transfer in crop improvement. Science 236:48-54.

Imamura, J. and H. Harada. 1980. Effects of abscisic acid and water stress on the embryo and plantlet formation in anther culture of *Nicotiana Tabacum* cv. Samsun. Z. Pflanzenphysiol. Bd. 100, S:285-289.

Inoue, M. and E. Maeda. 1981. Stimulation of shoot bud and plantlet formation in rice callus cultures by two-step culture method using abscisic acid and kinetin. Jap. J. Crop Sci. 50:318-322.

International Rice Research Institute. 1987. Annual Report for 1986. College, Philippines.

Jena, K.K. and G.S. Khush. 1984. Embryo rescue of interspecific hybrids and its scope in rice improvement. Int. Rice Genet. Newslett. 1:133-134.

Khush, G.S., K.C. Ling, R.C. Aquino, and V.M. Aguiero. 1977. Breeding for resistance to grassy stunt in rice. In: Proc. 3rd Intl. Cong., SABRAO, Canberra, Australia. Plant Breeding Papers 1-4(6). pp. 3-9.

Khush, G.S. and S.S. Virmani. 1985. Some plant breeding problems needing biotechnology. In: Biotechnology in International Agricultural Research, Proc. of Inter-Center Seminar on International Agricultural Research Centers (IARCs) and Biotechnology. 23-27 April 1984. Int. Rice Res. Inst., College, Philippines. pp. 51-63.

Mercy, S.T. and F.J. Zapata. 1986. Effect of sucrose on callus induction and plant regeneration in Taipei 309. Int. Rice Res. Newslett. 11(4):25.

Oono, K. 1985. Tissue culture applications in rice improvement. Paper presented at the Int. Rice Res. Conf. 1-5 June, 1985. Int. Rice Res. Inst., College, Philippines. 23 pp.

Ponnamperuma, F.N. 1984. Role of cultivar tolerance in increasing rice production on saline lands.

Quimio, C.A. and F.J. Zapata. 1988a. Diallel analysis of response to anther culture in rice. I. Callus induction. Submitted for publication.

Quimio, C.A. and F.J. Zapata. 1988b. Diallel analysis of response to anther culture in rice. II. Plant regeneration. Submitted for publication.

Scowcroft, W.R., R.I.S. Brettel, S.A. Ryan, P.A. Davies, and M.A. Pallota. 1987. Somaclonal variation and genomic flux. In: Plant Tissue and Cell Culture. Plant Biology, Vol. 3. C.E. Green, D.A. Somers, W.P. Hackett, and D.D. Biesbaer (eds.). Allan R. Liss, Inc., New York. pp. 275-286.

Scowcroft, W.R. and P.J. Larkin. 1982. Somaclonal variation: a new option for plant improvement. In: Plant Improvement and Somatic Cell Genetics. I.K. Vasil, W.R. Scowcroft, and K.J. Frey (eds.). Academic Press, London. pp. 159-178.

Scowcroft, W.R., S.A. Ryan, R.I.S. Brettell, and P.J. Larkin. 1985. Somaclonal variation in crop improvement. In: Biotechnology in International Agricultural Research. Proc. Inter-Center Seminar on IARCs and Biotechnology. 23-27 April, 1984. Int. Rice Res. Inst., Manila, Philippines. pp. 99-110.

Senadhira, D. 1988. Tidal wetland and problem soil rice. IRRI Internal Program Review. 3 pp.

Shimamoto, K., Y. Hayashi, and J. Kyozuka. 1986. High frequency of plant regeneration from protoplasts of rice (*Oryza sativa*) (Abstract). In: VI International Congress of Plant Tissue and Cell Culture. 3-8 August, 1986. Univ. of Minnesota, U.S.A. p. 161.

Snape, J.W. 1982. The use of doubled haploids in plant breeding. In: Induced Variability in Plant Breeding. Center for Agricultural Publishing and Documentation, Wageningen. pp. 52-58.

Swaminathan, M.S. 1986. Plant research and world agriculture. Plant Molecular Biology Reporter 4(1):1-17.

Swaminathan, M.S. 1987. China Trip Report. 21-24 July, 1987.

Terada, R., J. Kyozuka, S. Nishibayashi, and K. Shimamoto. 1987. Plantlet regeneration from somatic hybrids of rice (*Oryza sativa* L.) and barnyard grass (*Echinochloa oryzicola* Vasing). Mol. Gen. Genet.

Toriyama, K. and K. Hinata. 1985. Cell suspension and protoplast culture in rice. Plant Sci. 41:179-183.

Toriyama, K. and K. Hinata. 1986. Plant regeneration from protoplasts of anther callus in rice (Abstract). In: VI International Congress of Plant Tissue and Cell Culture. 3-8 August, 1986. Univ. of Minnesota, U.S.A. pp. 160.

Torrizo, L.B. and F.J. Zapata. 1986. Anther culture in rice. IV. The effect of abscisic acid on plant regeneration. Plant Cell Reports 5:136-139.

Tran, V.D. 1986. An overview of upland rice in the world. In: Progress in Upland Rice Research. Proceedings of the 1985 Jakarta Conference.

Wang, M.S. and F.J. Zapata. 1987. Somatic embryogenesis and plant regeneration in tissue culture of *Echinochloa glabrescens* Munro Ex. Hook F. J. Plant Physiol. 130:79-85.

Wang, M.S., F.J. Zapata, and D.C. de Castro. 1987. Plant regeneration from mature seed and inflorescence of wild rice (*Oryza perennis* Moench). Plant Cell Reports. 6:294-297.

Woo, S.C. and Associates. 1984. Salt-tolerant rice. Cited in Agricell Report 3:20.

Yamada, Y., Z.O. Yang, and D.T. Tang. 1986. Plant regeneration from protoplast-derived callus of rice (*Oryza sativa* L). Plant Cell Reports 5:85-88.

Zapata, F.J. and E.M. Abrigo. 1986. Plant regeneration and screening from long-term NaCl-stressed rice callus. Int. Rice Res. Newsletter. 11(4):24-25.

Zapata, F.J., R.R. Aldemita, L.B. Torrizo, A.U. Novero, S.K. Raina, and R.R. Rola. 1986. Anther culture of Basmati 370 at IRRI. A. Gamma ray-induced green plant regeneration. Int. Rice Res. Newslett. 11(4):22.

Zapata, F.J. and L.B. Torrizo. 1986. Heat treatment to increase callus induction efficiency in anther culture of IR42. Int. Rice Res. Newslett. 11(4):25-26.

Zapata, F.J., L.B. Torrizo, and R.R. Aldemita. 1985. Effect of conditioned medium on callus production and plant regeneration in rice anther culture. Int. Rice Res. Newslett. 10(5):14.

Zhao, C., K. Zheng, X. Sun, and X. Zu. 1985. Somaclonal variation in rice improvement. Agricell Rep. 4:6.

Zimny, J., B. Junke, and H. Lorz. 1986. Root derived *in vitro* culture of rice: plant regeneration from callus and cell suspension and direct gene transfer to protoplast. In: International Congress for Plant Tissue and Cell Culture. 3-8 August 1986. Univ. of Minnesota, U.S.A. p. 207.

Tissue Culture Techniques for Germplasm Improvement and Distribution

John H. Dodds
International Potato Center
Lima, Peru

Introduction

For many years, tissue culture has been applied to improve potato production by means of micropropagation, pathogen elimination, and germplasm conservation.[1,2,3] However, some of these techniques are still being refined and improved.[4,5] Intermediate-level technologies such as *in vitro* tuberization,[6] and embryo and anther culture are having some direct application on germplasm distribution and germplasm improvement.[7] In the case of sweet potato, tissue culture and rapid propagation have been applied in many countries. The most sophisticated technologies such as genetic engineering and protoplast fusion have potential to improve potato and sweet potato production although translation of that "potential" into reality will have to be carried out with appropriate care. This article will analyze the spectrum of technologies mentioned in this section and consider their impact on potato and sweet potato improvement and production.

IN VITRO GERMPLASM CONSERVATION

A number of tissue culture methods have been applied for conservation of potato and sweet potato germplasm *in vitro*; these include the use of growth-retarding compounds,[8] reduction in incubation temperature,[9] and, less commonly, freeze preservation by cryopreservation.[10,11] Most potato and some sweet potato programs apply tissue culture germplasm conservation to some extent; this may be the maintenance of a few genotypes used in a seed program, or it may be a major germplasm collection such as that at CIP with over 5,000 clonal accessions of potato and 2,500 of sweet potato.

A number of advantages exist for *in vitro* germplasm collections over that of field-maintained collections: (1) the material is available all year round, (2) it is protected from environmental and pathogen risks, and (3) it is relatively simple to produce multiple copies of the collection to maintain duplicates in different geographical locations.

(a) *Use of Growth Retardants*

A wide range of chemical growth retardants have been tested on *in vitro* potato and sweet potato plantlets. The objective in using these compounds is to lower the growth rate of the *in vitro* plantlets in order to lengthen the time between subcultures.

Maleic hydrazide (MH), an active compound in a number of commercially available growth retardants, has been shown to promote tuberization in cultured stem sections of *S. tuberosum* cv. British Queen.[12] Diamonozide (B995) is normally used extensively as a foliar spray on ornamental plants such as chrysanthemum and azalea; however, Humphries and Dyson reported a 10% increase in tuberization after spraying it on plants of *S. tuberosum* cv. Majestic;[11] it also retarded plant growth. Phenolic compounds such as transcinnamic acid (TCA) have been shown to stimulate tuberization from stem cuttings *in vitro*. Abscisic acid (ABA) is present in potato tubers and it is involved in the control of dormancy where it has been used to act as a natural growth retardant.

An alternative approach to use growth retardants has been increasing the osmotic pressure of the medium, by adding sucrose or the metabolically inactive sugar alcohols mannito and sorbitol, in order to reduce the water available to growing cultures.[14]

Changing the available carbon source, generally sucrose, can have a marked effect on growth rate, either as a nutritional factor or an osmotic factor. Increasing the sucrose concentration in the medium up to 8% is an effective method to retard growth; however, there are problems of high mortality rates after six months of storage with both potato and sweet potato. An alternative method of reducing growth is to include a sugar alcohol that cannot be metabolised such as sorbitol or mannitol in the medium. These additives are very effective for regulating *in vitro* plantlet growth.

(b) *Growth Regulation by Reduced Temperature Incubation*

Plants, like most organisms, live within a fairly restricted temperature range. Plant growth processes are under the control of a large number of regulatory enzymes. Biochemically, each of these enzymes has a well-defined temperature optimum, and this consequently results in a temperature optimum for growth of the plant. It follows, therefore, that if the *in vitro* plants are maintained at temperatures either significantly above or below this optimum, their growth will be restricted, and care must of course be taken not to overstress the plant. If temperatures fall much below 3°C in potato and 15°C and below in sweet potato, cold damage will kill the

plants. Likewise temperatures above 28°C in potato and 40°C in sweet potato will cause excessive heat stress. Within this range of temperatures, plants will survive and grow; however, the time period between subcultures changes markedly on either side of the 6°C mark in potato and 18°C in sweet potato. The optimal growth inhibition temperature for potato plantlets while maintaining high viability is 6°C,[15] whilst 18°C is optimal for sweet potato.

Although some experiments were performed to study survival of material maintained below 0°C down to −12°C, the success of these experiments was limited. However, some success has been achieved by dropping to extremely low temperatures (−196°C) using techniques of cryopreservation.[16,17,18,19,20]

(c) *Cryopreservation of Germplasm*

A comprehensive review by Withers (and references cited therein) has discussed fully all aspects of the cryopreservation of plant cell, tissue, and organ cultures.[21] Only the basic principles and methodology of freeze-preservation will be discussed in the following sections, since any attempt to briefly survey and discuss the literature already available would go beyond the scope of this paper.

The successful cryopreservation of a particular plant tissue requires that damage caused by ice crystal formation within the individual cells of the tissue is either prevented or minimized. This has led to the adoption of two basic approaches to cryopreservation: either ultrarapid freezing or slow/stepwise freezing. The process of rapid freezing results in the formation of ice crystals within the cells which are of microscopic size, and which do not disrupt the internal organelles and membranes. However, thawing must be carried out rapidly enough to prevent recrystallization. Henshaw[22] has reported the use of this method for successfully freeze-preserving potato shoot-tips. Slow or stepwise freezing has been applied to many tissue culture systems and depends upon extracellular freezing for protection against cellular damage. As cells are cooled, the surrounding liquid (either intercellular fluid or extracellular culture medium) will eventually freeze due to ice nucleation. However, the intracellular fluid will not yet be frozen, and so a process of cellular dehydration will be initiated due to the water vapour pressure deficit established by extracellular ice formation. The concentration of intracellular solutes resulting from dehydration then further depresses the freezing point of the cell contents. This process of gradual removal of intracellular water is termed "protective-dehydration," and effectively prevents ice formation in the cytoplasm or vacuole. However, to date the success rate of

cryopreservation with potato germplasm is generally low, and little is known of the genetic damage that may be caused by the technique. At the present time, to my knowledge, no institution is using cryopreservation as a standard method for germplasm conservation. However, many institutions are currently using growth inhibitors and reduced incubation temperatures.

PATHOGEN ELIMINATION

The use of meristem culture coupled to thermotherapy or chemotherapy has for many years been an important component of pathogen elimination to potato and sweet potato seed programs. The techniques can not only increase marketable yield by producing pathogen-tested plants, but also facilitates germplasm exchange by removing virus infections which allow compliance with quarantine regulations.[23] The establishment of pathogen elimination programs has greatly aided the international distribution of both potato and sweet potato germplasm, and thus indirectly, potato and sweet potato production. It is worth noting, however, that experiments are in progress in several institutions to streamline pathogen (virus) elimination procedures.[24] The basic procedures are as follows:

Thermotherapy

Thermotherapy at elevated temperature (37°C) does not eliminate the potato spindle tuber viroid (PSTV). PSTV consists of a single-stranded, ring-shaped RNA that is twisted in the form of a supercoil. In this form it is resistant to nucleases. Elevated temperature, far from decreasing the concentration of the viroid, favors its multiplication.[25] Therefore, a first test for PSTV should be carried out at the end of the thermotherapy period.

A method that permits the eradication of PSTV[26] is based on the observation that in plants grown at low temperatures, the viroid concentration is low. In an experiment, plants were grown at 8°C for four months. Then apical domes were excised. Thirty percent of the plantlets regenerated were free of PSTV, even in the second tuber generation. A clear (negative) relationship between meristematic explant size and eradication success was observed.[27]

This method, however, is not suitable as a routine technique, since it is time-consuming and costly. It may be useful in specific cases in which a valuable clone is heavily infected and no pathogen-tested material is available.

Experiments carried out with different virus host systems have shown that treatment of plants with elevated temperatures (ther-

motherapy) leads to a reduction in virus concentration (titre) in the plant.[28] Different reasons have been given to explain this phenomenon; probably not one alone, but a combination of several may be the cause for reduction in virus titre. These can include competition for sites of synthesis of nucleic acids and proteins between the fast-dividing host cells and the virus particles, which may lead to a change in the balance between synthesis and degration of virus particles. Also, the nucleic acid of the virus, the carrier of its genetic information, is usually protected from attacks by degrading enzymes because of a coat made of many protein subunits. At elevated temperatures the linkage between these subunits becomes weaker; temporary holes may open and permit the attack of nucleases, leading to inactivation of virus and decrease of virus concentration.

Thermotherapy has been applied to dormant potato tubers. A reduction of virus concentration, mainly of potato leaf roll virus (PLRV) has been observed. However, elimination was not generally achieved except for PLRB.

Thermotherapy applied to the whole plant as well as to sprouted tubers followed by meristem culture has been successfully used for elimination of many viruses in potato.[29,30]

In the standard procedure, best results have been obtained when the plant is decapitated before introduction into thermotherapy, and axillary buds grown while undergoing heat treatment. A daily temperature regime of 36°C for 16 hours and 30°C for 8 hours and continuous light of high intensity (10,000 lux) improved elimination rates. Plants are kept under these conditions for 4 weeks. From axillary buds as well as apical buds, meristems are isolated and cultivated.

Chemotherapy

As an alternative to thermotherapy, chemotherapy has been tried in potato. A nucleoside analogue, Virazole, known for its broad spectrum against animal DNA and RNA viruses, has shown variable results when applied to the potato plant as spray or in hydroponic culture followed by meristem culture. Preliminary results show some promise when culturing excised meristem tips in the presence of 100 ppm Virazole in the medium.

However, some reports indicate that antiviral chemicals may cause mutations in the plant. Therefore, at the present time, thermotherapy is definitely the preferred method of pretreatment prior to meristem culture.

Meristem Isolation and Culture

The active growing point of the plant shoot is the meristem. It is a small region composed of rapidly dividing (meristematic) cells.

The dome of a shoot apical meristem contains the truly meristematic cells and is surrounded by leaf primordia and primary leaves. Since the more differentiated vascular tissues are found distant from the meristem (towards the older tissue of the stem), the vascular elements of the leaf primordia are still incipient, and have not yet made contact with the main strand of the vascular system in the stem. Therefore, virus particles, which may be present in the vascular system, can reach the meristematic region of the apex only through cell-to-cell movement; a slow process. This is one of the main reasons why in a virus-infected plant, virus concentration decreases acropetally toward the meristem of both the apical and the axillary buds.

Isolation of the apical portion, called the meristem tip, under aseptic conditions and its culture on an adequate aseptic nutrient medium, leads to the development of plantlets. This developmental sequence, in principle, follows a pattern similar to that in the normal plant growth. The cells of the meristem divide and the differentiation of new tissues continues. The nutrition of the excised portion of the plant is supplied by the artificial medium. This technique, called meristem culture, was first applied for virus eradication some 30 years ago by Morel and Martin (1952) on Dahlia, and leads to pathogen-free plants.

The aseptic dissection of the meristem is a delicate process and requires many hours of practice. The sequence of dissection is shown photographically (Figure 1) and is carried out as follows:

Stems are cut from the plant that has just undergone thermotherapy into segments each containing one node with its axillary bud. The leaves are carefully removed. The stem segments are disinfected for 30 seconds in 70% alcohol, followed by 2.5% calcium hypochlorite for 15 minutes. Then the stems are washed four times 5 minutes each with sterile distilled water to remove excess hypochlorite.

Under a binocular dissecting microscope, the leaflets surrounding the growing point are removed until only the apical dome and a few leaf primordia remain. The dome and two leaf primordia are excised and transferred to meristem culture medium. The excised apical dome is transferred weekly to fresh medium. After 6-8 weeks, the small plantlets are subcultured for further growth and micropropagation.[31]

After regeneration from the cultured meristems, plants are tested (indexed) to detect any persisting virus infections.

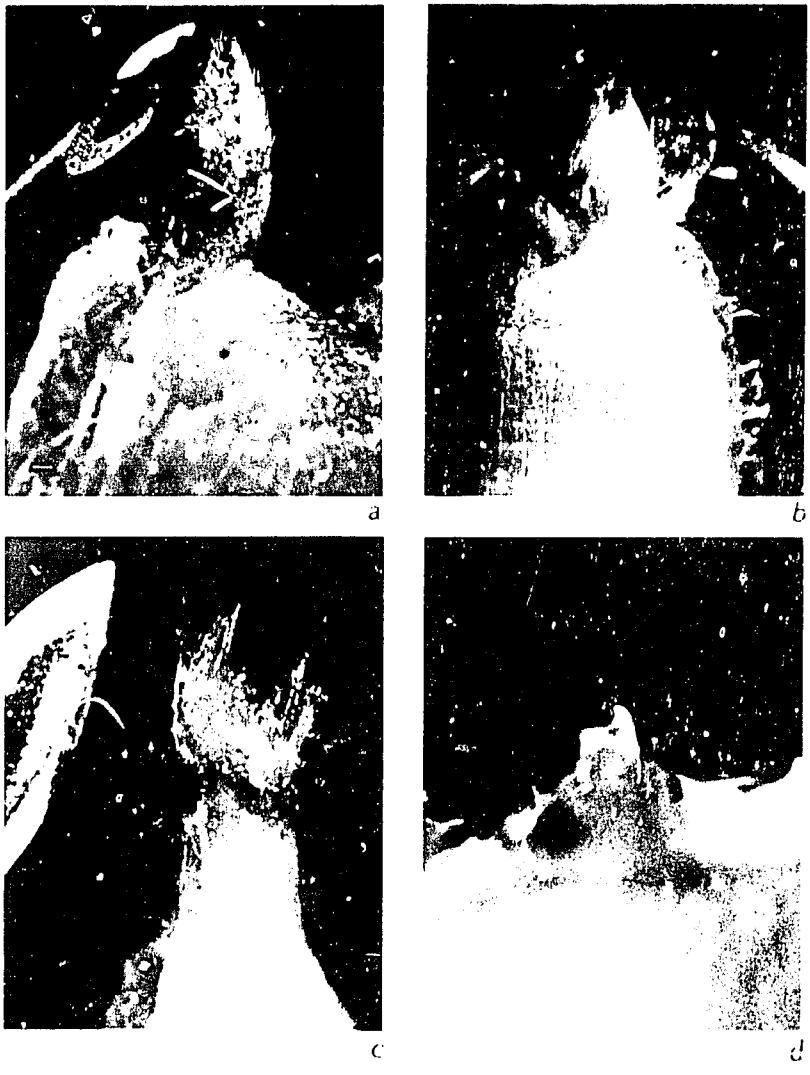


Figure 1 Photographic sequence of meristem dissection:

(a) The isolated surface-sterilised bud. (b & c) Various stages of the dissection process where primary leaves are removed. (d) The fully dissected meristem with two leaf primordia.

IN VITRO MICROPROPAGATION

Most potato and sweet potato seed programs use as starting material, *in vitro* pathogen-tested plantlets produced using cleanup methods described above. The initial stages of the seed program can make use of varying amounts of *in vitro* micropropagation depending on the size, location, and facilities of the program. The basic methods used, however, are very similar in most institutions and are based on the rapid growth on solid or liquid culture media of single-node cuttings or stem explants. The basic micropropagation methods used at CIP and many other institutions are as described below:

(a) *Growth of Single-Node Cuttings*

Single nodes with leaves are excised from small *in vitro* plantlets; in some genotypes the large leaves are carefully removed. The removal of the leaves ensures uniform growth of the newly developing shoots. If large leaves are left on the single-node pieces, it is believed that hormones from the senescing leaf can inhibit growth of the newly developing shoot. Each node is then inoculated onto the surface of agar-solidified medium.[32] The axillary bud quickly grows out (Figure 2), and in 3-4 weeks a plantlet with six or seven more nodes becomes available for subculture. Figure 2 shows micropropagation of both potato and sweet potato cultures.

(b) *Liquid Shaken Cultures*

This type of culture is performed to rapidly produce large numbers of nodes for subsequent nodal cutting preparation.

In vitro plantlets are cut into stem cuttings each with three to four nodes; the large leaves are removed. Each stem piece is placed in 15 ml of liquid medium[33] and the flasks are shaken (80 rpm). After 2-3 weeks of rapid growth, each flask contains 60 to 70 nodes in case of potato, 20-25 in the case of sweet potato. In the case of sweet potatoes, the plants are of such a size that 20-30 nodes/flask would be an upper limit. Once a suitable number of small plantlets have been produced, single-node cuttings are prepared and grown *in vitro* until they can be transferred to nonsterile conditions, i.e., transplanted into beds or pots for tuber production (see Figure 2).

(c) *Rooting and Transfer to Nonsterile Conditions*

After removal of the large leaves, between 16-25 excised single nodes of *in vitro* plantlets are placed on agar-solidified medium in a suitable container. When the plantlets are 3-5 cm. high and have developed a good root system (Figure 2), they are ready for

transplanting into pots or beds containing a suitable high-organic mixture (Figure 2).

These basic technologies have had a dramatic effect on the amount of high-quality seed produced in many programs. In recent years, a number of institutions have been using *in vitro* potato plantlets transplanted at high densities (i.e., 100/m²) to produce small, high-quality tubers for subsequent field multiplication. Small storage roots have also been produced in sweet potatoes, however, at lower planting densities.

IN VITRO TUBERIZATION (Potato)

In recent years, interest has developed in many countries on the induction of potato tubers under *in vitro* conditions. Several different methods are available to bring about the induction process.[34,35] At CIP, we have developed a rapid, cost-effective method that involves the addition of CCC (Chlorocholine Chloride) and sucrose to the liquid medium used for propagation.[36] We have shown that this technique is not only rapid and efficient but is applicable to a wide range of potato genotypes.

Figure 3a shows diagrammatically the basic methods used for the induction process. Programs in many developed and developing countries have started *in vitro* tuberization programs. The tubers are normally utilized in two different ways. Firstly, for distribution of germplasm either nationally or internationally; secondly, these small tubers can be used as an additional component to the standard methods of rapid propagation used in a seed tuber production program.

At CIP, the use of *in vitro* tubers offers some advantages for distribution of germplasm. The tubers are produced under *in vitro* conditions from pathogen-tested plantlets and thus comply easily with international quarantine requirements. However, unlike *in vitro* plantlets, if the package is delayed in transit, there are less problems of losing the material. Figure 4a shows freshly packed *in vitro* tubers for export whilst Figure 4b shows the same tubers after eight weeks at 22°C packed in the same way as distributed tubers; clearly, even after this period of time, material can still be utilized. There are disadvantages, however—the unit cost of production of an *in vitro* tuber is significantly more than that of an *in vitro* plantlet. For that reason, CIP is only using this type of distribution to certain locations where normal transit times are prolonged.

The unit cost analysis should also be used for seed programs. Although there are many advantages to using *in vitro* tubers in a

seed program, care must be exercised in deciding where it is useful in terms of a cost-benefit analysis. For example, in areas where *in vitro* plantlets can be transplanted all year round, there is little point in producing *in vitro* tubers. In the case of sweet potatoes, attempts to produce *in vitro* storage roots have so far been unsuccessful.

RECEIPT AND MULTIPLICATION OF MATERIAL BY NATIONAL PROGRAMS

In recent years, significant extra training has been carried out through CIP's global regional network to enable national programs to receive and multiply *in vitro* materials. Figures 5a and 5b show the number of people trained by CIP in tissue culture techniques over a ten-year period and the number of *in vitro* clones distributed. National programs now normally prefer to receive new clones as *in vitro* materials so as to be able to quickly multiply the new material and get it to farmers. In some cases, i.e., Vietnam, the tissue culture propagation has even reached small farmer level.

USE OF CONTRACT RESEARCH IN BIOTECHNOLOGY FOR GERMPLASM IMPROVEMENT

In collaboration with the Biochemistry Department at Louisiana State University, CIP has developed a project to insert synthetic genes into potato and sweet potato plants by genetic engineering. In this project using *Agrobacterium* plasmid vectors,[37,38] we have established a system of gene transfer the same as is now used by several laboratories worldwide, including laboratories in Europe, the United States, Brazil, Mexico, and India. The primary objective of the project is to enhance the nutritional value of the potato and sweet potato by obtaining the supplementary production of a synthetic protein rich in essential amino acids. The synthetic protein is produced from a gene that has been synthesized artificially in the LSU laboratory.

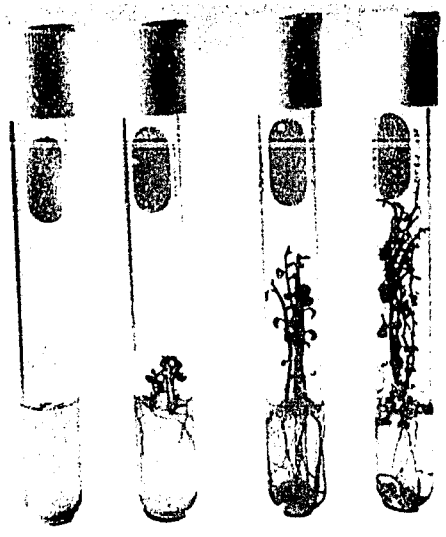
Through this research collaboration, inserting the synthetic gene into potato and sweet potato plants has been successful. In the case of potato, evidence has also been obtained that the gene is transcribed and produces a corresponding messenger RNA (MRNA) molecule, which is then translated in the plant to produce the synthetic protein[39] (Figure 6 shows these translational steps). This synthetic gene project has shown that the technology now exists to genetically engineer genes into the potato and perhaps into sweet potato. However, much more research, as well as the appropriate international legislative measures, will be needed before these genetically engineered plants can be released to national programs.

On the basis of the success with the synthetic protein gene, CIP is directing attention to the possible use of genetic engineering to give the potato or sweet potato plant resistance to pests and diseases.[40] Through collaborative links, CIP hopes to genetically engineer plants for resistance to viroid and virus infections by inserting gene sequences that will intertere with virus and viroid replication. This approach would allow potato plants to be grown for more generations in the field before degeneration of seeds occurs.

Collaborative research is also being developed to attempt to engineer resistance in the potato to disease caused by bacteria such as *Pseudomonas* and *Erwinia*. For these experiments, we are using genes that have been purified and that code for proteins with known, potent antibacterial activity. A genetic engineering approach to bacterial disease resistance may allow us to develop clones with resistance to both *Erwinia* and *Pseudomonas* in the same clone without major changes of the existing agronomically acceptable characters of that clone.

Conclusions

The range of sophisticated techniques available in tissue culture is clearly a broad one. Some of these techniques are already having a direct and significant impact on potato production, and their use is widespread. Other techniques are still at a developmental stage; they offer great potential for direct or indirect improvement of potato production. However, time will be needed before the possible benefits of these types of research are transferred to farmers' fields.



a

b

Figure 2A: Potato

(a) Growth of single-node cuttings on solid media. (b) Shaken cultures with stem segments in liquid medium containing GA.

Figure 2B: Sweet Potato

(c) Plastic box with individual rooted plantlets ready for transfer to pots or seedbeds. (d) In vitro plantlets a few days after transplant to jiffy pot.

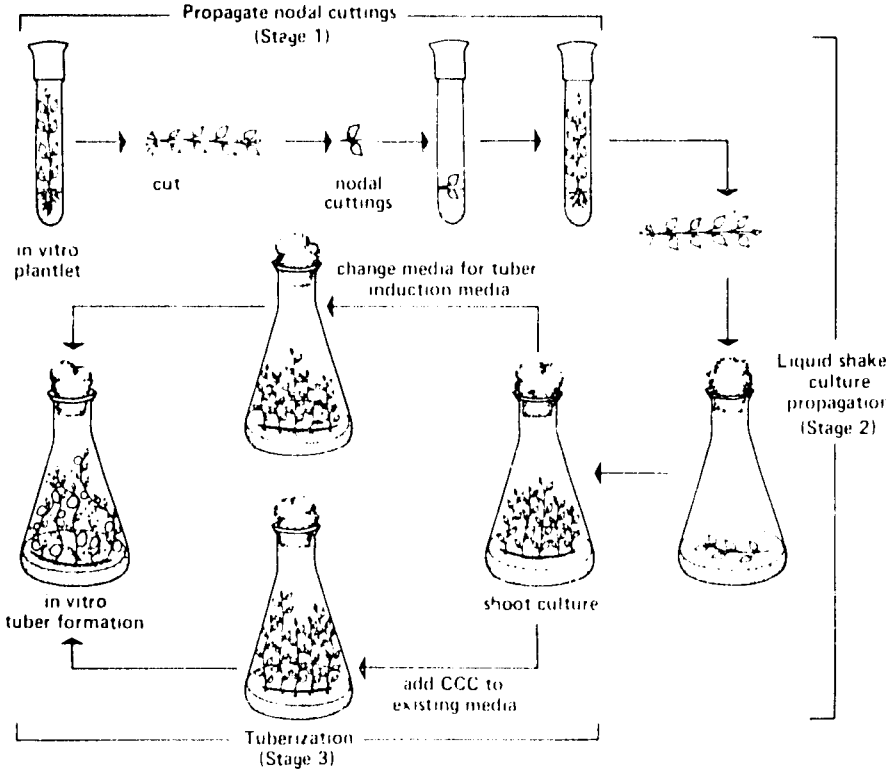
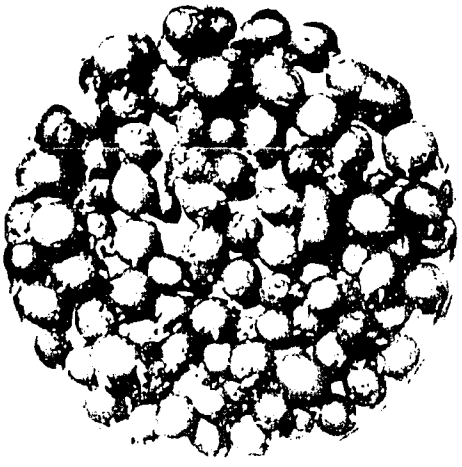
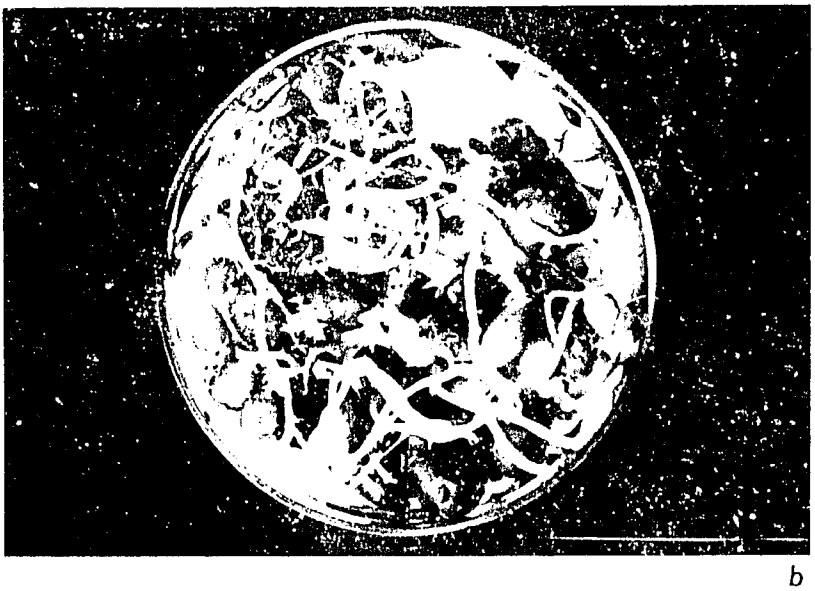


Figure 3 (a) Schematic representation of the basic methodology used for in vitro tuber induction at CIP.





b

Figure 4 (a) Sterile petri dishes containing in vitro tubers; these dishes are packed with cotton wool or vermiculite and shipped air freight to their destination. (b) A similar group of in vitro tuber after four months of storage at room temperature. Although sprouts are long, plantlets can be recued from this material.

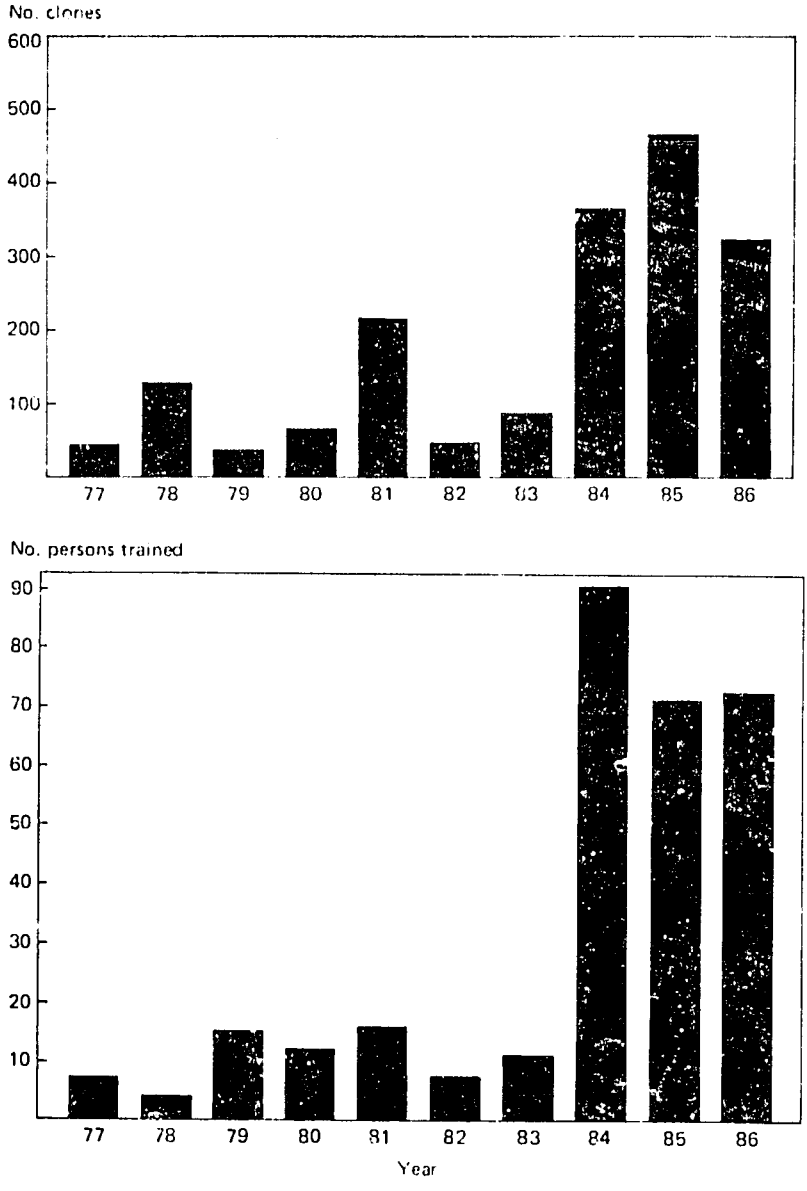


Figure 5 (a) Number of in vitro clones distributed to National Programs between 1977 and 1986. (b) National collaborating scientists trained by CIP in micropropagation and rapid multiplication techniques between 1977 and 1986.

A

AATTCGGGGATCGTAAGAAATGGATGGATCGTCATCCATTTCTTCATCCATTTCTTAC
GCCCCTAGCATTCTTTACCTACCTAGCAGTAGGTAAAGAAGTAGGTAAAGAATG

GATCCATCCATTTCTTAAGAAATGGATGAAGAAATGGATGACGATCCATCCATTTCTT
CTAGGTAGGTAAAGAATTCTTTACCTACTTCTTTACCTACTGCTAGGTAGGTAAAGAA

CATCCATTTCTTCATCCATTTCTTACGATCAAGAAATGGATGAAGAAATGGATGAAGA
GTAGGTAAAGAAGTAGGTAAAGAATGCTAGTTCTTTACCTACTTCTTTACCTACTTCT

AATGGATGAAGAAATGGATGCATCCATTTCTTAAGAAATCGATGAAGAAATGGATGAA
TTACCTACTTCTTTACCTACGTAGGTAAAGAATTCTTTACCTACTTCTTTACCTACTT

GAAATGGATGACGATCGATCGTAAGAAATGGATGACGATCGATCCATTTCTTACGATC
CTTTACCTACTGCTAGCTAGCATTCTTTACCTACTGCTAGGTAGGTAAAGAATGCTAG

CCCG
GGGCTTAA

B

GlyAspArgLysLysTrpMetAspArgHisProPheLeuHisProPheLeuThrIleHisProPhe
LeuLysLysTrpMetLysLysTrpMetThrIleHisProPheLeuHisProPheLeuHisProPheLeuThr
IleLysLysTrpMetLysLysTrpMetLysLysTrpMetLysLysTrpMetHisProPheLeuLysLysTrp
MetLysLysTrpMetLysLysTrpMetThrIleAspArgLysLysTrpMetThrIleHisProPheLeuThr
IlePro

Figure 6 (a) A synthetic DNA sequence coding for a protein rich in essential amino acids (SP47). This gene can be translated in either direction A or B to produce 2 synthetic proteins (1) and (2) whose amino acid composition is shown in (b).

References

1. Roca, W.M., J.E. Bryan, and M.R. Roca. 1979. Tissue Culture for the International Transfer of Potato Genetic Resources. American Potato Journal 56:1-10.

2. Roca, W.M., N.O. Espinoza, M.R. Roca, and J.E. Bryan. 1978. A Tissue Culture Method for the Rapid Propagation of Potatoes. American Potato Journal 55:691-701.

3. Schilde-Rentschler L., N.O. Espinoza, R. Estrada, and R. Lizarraga. 1982. *In vitro* storage and distribution of potato germplasm. 5th International Plant Tissue Culture Congress. Japan. E. Fujiwara.

4. Espinoza, N.O., R. Estrada, P. Tovar, J.E. Bryan, and J. Dodds. 1984. Tissue Culture Micropropagation, Conservation and Export of Potato Germplasm. Specialized Technology Document. International Potato Center, Lima, Peru. 20 pp.

5. Dodds, J.H., D. Silva-Rodriguez, and J.E. Bryan, 1986. Transport, Receipt and Propagation of *In Vitro* Potato Plantlets. International Potato Center, Lima, Peru.

6. Tovar, P., R. Estrada, L. Schilde-Rentschler, and J.H. Dodds. 1985. Induction and Use of *In Vitro* Potato Tubers. CIP Circular Vol. 13 (4).

7. Sonnino, A. 1984. Anther Culture and Its Uses in Potato Breeding. In: Innovative Methods for Propagating Potatoes. International Potato Center, Lima, Peru, 319-335.

8. Westcott, R.J. 1981. Tissue Culture Storage of Potato Germplasm. Use of Growth Retardants. Potato Research 24:343-352.

9. Schilde-Rentschler, L. 1979. In: Report of the Planning Conference on the Exploration, Taxonomy and Maintenance of Potato Germplasm III. International Potato Center, Lima, Peru, 182-193.

10. Grout, B.W.W. and G.G. Henshaw. 1978. Freeze Preservation of Potato Shoot-tip Cultures Ann. Bot. 42:1227-1229.

11. Towill, L.E. 1981. Survival at low temperatures of shoot-tips from cultivars of *Solanum Tuberosum* Group Tuberosum. Cyo-Lett. 2,373-382.

12. Harney, M.A., M.P. Crawley, and P.E.M. Clinch. 1966. The Effect of Gr—Regulators on Tuberization of Cultived Stem Segments of *Solanum Tuberosum*. Eur. Pot. J. 9 146-151.

13. Humphres, E.C. and E.C. Dyson. 1967. Effect of Growth RegulatorN-Dimethyl Amino Succinamic Acid on Potato Plants in the Field. Eur. Pot. J. 10 116-126.

14. Cram, W.J. 1984. Mannitol Transport and Suitability as an Osmoticum in Root Cells. Physiol. Plant 61:396-404.

15. Henshaw, G.G., J.P. O'Hara, and R.J. Westcott. 1980. In: Tissue Culture Methods for Plant Pathologists (ed. by D.S. Ingram and J.P. Helgeson).

16. Withers, L.A. 1985. Cryopreservation of Cultivated Cells and Meristems. In: Cell Culture and Somatic Cell Genetics of Plants, Vol. 2 Academic Press Inc. pp. 254-316.

17. Bajaj, Y.P.S. 1979. Technology and Prospects of Cryopreservation of Germplasm. Euphytica 28:27-285.

18. Kartha, K.K. 1985. Meristem Culture and Germplasm Preservation. In: Cryopreservation of Plant Cells and Organs (Ed. K.K. Kartha) CRC Press Inc. pp. 276.

19. Henshaw, G.G., J.F. O'Hara, and J.A. Stamp. 1985. Cryopreservation of Potato Meristems. In: Cryopreservation of Plant Cells and Organs (Ed. by K.K. Kartha) CRC Press Inc. pp. 159-170.

20. Grout, B.W.W. and G.G. Henshaw. 1980. Structural Observations on the Growth of Potato Shoot-tip Cultures after Thawing from Liquid Nitrogen. Ann. Bot. 46, 243-248.

21. Withers, L.A. 1980. Low Temperature Storage of Plant Tissue Cultures. In: Advances in Biochemical Engineering. Plant Cell Cultures II (ed. by A. Fiechter) Springer-Verlag Berlin Heidelberg pp. 101-150.

22. Henshaw, G. 1975. Technical Aspect of Tissue Culture Storage for Genetic Conservation. In: H. Frankel and J.G. Hawkes. Crop Genetic Resources for Today and Tomorrow. CUP. 349-358.

23. Hewitt, W.B. and L. Chiarappa. 1977. Plant Health and Quarantine in International Transfer of Genetic Resources, IBPGR. CRC Press Inc.

24. Slack, S. 1987. Use of *in vitro* thermotherapy for virus eradication. Amer. Pot. J. (in press).

25. Sanger, H.L. and K. Ramm. 1975. Radioactive labelling of viroid RNA. pp. 230-253. In: Markhan *et al.* (Eds.) Modification of the information content of plant cells. Proceedings of 2nd John Innes Symposium, Norwich, U.K.

26. Lizarraga, R.E., Salazar, L.F., Roca, W.H., and L. Schilde-Rentscheler. 1980. Elimination of potato spindle tuber viroid by low temperature and meristem culture. Phytopathology 70:754-755.

27. Lizarraga, R.E. and L.F. Salazar. 1982. Effect of meristem size on eradication of potato spindle tuber viroid. pp. 118-119. In: Hooker, W.J. Research for the potato in the year 2000. International Potato Center, Lima, Peru. 199 pp.

28. Kassanis, B. 1957. The use of tissue culture to produce virus free clones from infected potato varieties. Annals Applied Biology 45:422-427.

29. Stace-Smith, R. and F.C. Mellor. 1970. Eradication of potato spindle tuber virus by thermotherapy and axillary bud culture. Phytopathology 60:1957-1958.

30. Pennazio, S. and P. Redolfi. 1973. Factors affecting the culture *in vitro* of potato meristem tips. Potato Research 16:20-29.

31. Espinoza, N.O. *et al.*

32. *Ibid.*

33. *Ibid.*

34. Towill, L.E. 1981. Survival at low temperatures of shoot-tips from cultivars of *Solanum Tuberosum* Group Tuberosum. Cyo-Lett. 2, 373-382.

35. Estrada, R., P. Tovar and J.H. Dodds. 1986. Induction of *In Vitro* Tubers in a Broad Range of Potato Genotypes. Plant Cell, Tissue and Organ Culture 7:3-10

36. Tovar, P. *et al.*

37. Jaynes, J.M., M.S. Yang, N. Espinoza, and J.H. Dodds. 1980. Plant Protein Improvement by Genetic Engineering: Use of Synthetic Genes. Trends in Biotechnology. December 1986 pp. 314-320.

38. Dodds, J.H. 1987. CIP Collaborative Research in Tissue Culture and Genetic Engineering of the Potato. CIP Circular 15:1-5.

39. Espinoza, N.O., S.F. Yang, J.M. Jaynes, and J.H. Dodds. 1987. Genetic Engineering of the Potato. Scientific American (in press).

40. Jaynes, J.M., L. Xanthoporilos, L. Destefano, and J.H. Dodds. 1987. Increasing Bacterial Disease Resistance in Plants Utilizing Antibacterial Genes from Insects. Bioessays (in press).

Impact of Somaclonal Variation on Plant Improvement and IARC/Private Sector Collaborative Research

William R. Scowcroft
Biotechnica Canada Inc.
Calgary, Canada

Introduction

The original concept of somaclonal variation (Larkin and Scowcroft, 1981) has gained widespread acceptance and has been the subject of numerous reviews (see Scowcroft, 1985; Larkin, 1987). This variation, which arises as a consequence of tissue culture, has been found in essentially all plant species that have been regenerated from tissue culture. Recently, research has been directed at understanding the cause of somaclonal variation rather than mere documentation of the phenomenon and, significantly, to integrate the findings into plant breeding.

Genetic and molecular analysis is providing some understanding of the events that give rise to somaclonal variation. In maize, a new fully functional electrophoretic variant at the alcohol dehydrogenase locus has been found among somaclones and subsequently characterized as resulting from a single nucleotide substitution. In wheat, variants have been analyzed that affect traits such as height and alcohol dehydrogenase synthesis. Somaclonal analysis in maize and alfalfa has shown that cell culture greatly enhances the activation of transposable elements. For plant improvement, the enhanced frequency of genomic rearrangements during culture provide a new option to introgress alien genes from wild relatives into domesticated crops, as well as provide genetic variability for genetic improvement of asexually propagated crops.

Several critical analyses have been conducted at the genetic, cytogenetic, and molecular levels. These analyses reveal that somaclonal variation can give rise to mutants that behave as classical Mendelian mutants, genetic changes that affect characters under the control of multigene families, those that affect polygenically determined traits, and those that give the appearance of resulting from activation of transposable elements. Polyploid and aneuploid variants occur frequently, as do chromosomal rearrangements such as duplications, deficiencies, translocations, and other interchanges.

This following discussion provides some details of several recently analyzed somaclonal variants. They give some insight into the

nature of the genetic changes that occur during tissue culture. Somaclonal variation has been integrated into a number of plant breeding programs at national, international, and private corporation levels.

Mendelian Mutants

The genetic analysis of plants regenerated from tissue cultures of diverse species such as rice, wheat, maize, lettuce, tobacco, tomato, celery, and rapeseed demonstrates the occurrence of classical point mutations. Many of these mutants are allelic to other known mutants. In many cases, there is substantial evidence that these mutations occurred during the tissue culture phase because sister plants regenerated from the same callus were nonmutant. One such mutant, which has been analyzed at the nucleotide level, arose in regenerated maize plants that were screened for *isozyme variants at the alcohol dehydrogenase (*Adh1*) locus (Brettell *et al.*, 1986a).

Maize Alcohol Dehydrogenase

Plants regenerated from an F_1 heterozygous for the allelic variants *Adh1-S* and *Adh1-F* were screened for *isozyme variation by electrophoresis. Among 645 primary regenerants, several showed presumptive variation, but only one (*Adh1- Usv*) was stably transmitted in subsequent seed generations. This mutant allele produced a functional enzyme with a slower electrophoretic mobility than either one of its progenitor alleles. The mutant allele was subsequently cloned using an ADH genomic DNA probe derived from the *Adh1-S* allele. Subsequent restriction endonuclease analysis showed no major sequence reorganization and also confirmed that the mutant allele was derived from the parental *Adh1-S* allele rather than the fast-migrating *Adh1-F* allele.

Sequencing of the entire mutant *Adh1-Usv* gene revealed a single base change in the coding triplet of exon 6 immediately adjacent to the junction with intron 6. This substitution, where a thymine residue replaced adenine, translated into a polypeptide sequence where a glutamic acid residue was replaced by valine. This, in turn, significantly affects the charge on the ADH protein resulting in slower electrophoretic mobility.

The spontaneous rate of mutation at the *Adh* locus is very low, i.e., 10^{-5}–10^{-6}. Although only a single mutant has been found as a result of somaclonal variation, the frequency at which it occurred was in the neighborhood of 10^{-3}.

Somaclonal Mutants and Chromosomal Rearrangement

One of the most frequent causes of somaclonal variation is chromosomal rearrangement. Larkin (1987) documents numerous examples of recorded evidence for deletions, chromosome fusion, and interchanges. The consequences of such rearrangements can result in mutations that affect the phenotypic expression of one or more genes.

In wheat, Larkin *et al.* (1984) reported extensive genetic variability among somaclonal regenerants and simultaneously Karp and Maddock (1984) reported frequent occurrence of chromosome variation in wheat plants regenerated from tissue culture. In a recent analysis, Davies *et al.* (1986) analysed the progeny of more than five hundred plants regenerated from a wheat cultivar for potential variation in the expression of alcohol dehydrogenase-1 isozymes. *Adh1* in hexaploid wheat is complicated by the fact that the locus is triplicated and that functional ADH isozymes are dimers formed by random association of each of the three different monomers.

Seventeen ADH variants were identified and analyzed. Simple aneuploidy for chromosomes 4A or 4D accounted for 13 of these variants and the remaining four were euploid. A genetic and cytogenetic analysis of three of the mutant euploid somaclonal variants indicated that three different types of chromosomal rearrangements had occurred. The translocation mutant, *SVI*, resulted from a translocation of part or all of the chromatin from 4A to the short arm of chromosome 7B. The translocated segment carried the *Adh-A1*, *Gail* (gibberellic acid insensitive) and *Rht1* (reduced height) loci. This stable, nonreciprocal translocation line now has four doses of the *Adh-A1* and *Rht1/Gail* loci rather than the normal two. The phenotypic consequences of this are an altered alcohol dehydrogenase isozyme pattern and plants that are substantially reduced in height

A second mutant, *SV5*, was also shown to be the result of a nonreciprocal translocation. A segment of the short arm of chromosome 3B had been translocated to chromosome 4A resulting in the loss of function of the *Adh-A1*, *Rht1* and *Msic* (male sterility) loci.

The third mutant, Iso 4A, presumably resulted from breakage and centric fusion to result in an iso-chromosome. The phenotype reflected effects of increased dosage of both *Adh-A1* and *Rht1* genes.

The reason why cell and tissue culture results in an increased frequency of chromosomal aberration is yet to be explained. Several authors, but most notably Benzion *et al.* (1986), argue that late-replicating heterochromation "may occasionally replicate so late in mitosis of cultured cells that bridge formation and subsequent chromosome breakage occurs at anaphase." This would lead to a breakage-fusion-bridge cycle with consequent genetic affects such as deletions and chromosome interchanges.

Modified Gene Copy Number

Plant genomes can be large, with as much as 60% of the genome composed of repeated DNA. Repeated DNA can have a reiteration frequency per sequence as high as $10^5 - 10^6$ and can be clustered as tandem arrays or dispersed throughout the genome.

The ribosomal RNA (rRNA) genes of plants represent such highly repeated DNA sequences. Brettell *et al.* (1986b) examined the consequences of somaclonal variation in regenerated triticale plants by screening for possible perturbation of rRNA genes.

From among 192 regenerated plants of triticale screened by southern hybridization with a rDNA spacer probe, one variant displayed substantial perturbation. This variant showed an 80% reduction in the rDNA spacer sequences of rye chromosome 1. The sequence depletion, detected initially by the rDNA spacer probe, also had a substantially reduced level of C-band staining as revealed by cytological analysis. This rDNA depleted locus is the site of the nucleolar organizer in the rye genome component of triticale.

There is increasing evidence that somaclonal variation can lead to depletion or amplification of gene sequences. Peerbolte et al. (1987) established that an introduced DNA sequence in a tobacco cell line which resulted from transformation with an *Agrobacterium tumefaciens* construct, underwent modification during subsequent tissue culture cycles. Changes included depletion and rearrangement of the sequence. In addition, *in vitro* selection has resulted in genetic tolerance to antimetabolites, such as herbicides. In cases such as glyphosate tolerance in Petunia and phosphinotricin tolerance in alfalfa (Donn *et al.*, 1984), the tolerance arose as a result of amplifications of specific genes coding for an enzyme product which is the target for the herbicide in question.

"New" Genes from Somaclonal Variation

The question is frequently put, "Among the array of somaclonal variants derived from tissue culture, are the mutants the result of changes at preexisting loci, or do entirely new mutants occur?" Certainly, the latter is true, but the evidence for entirely new mutants is, at best, only circumstantial.

Some genetic events, which occur at a very low rate spontaneously, occur far more frequently during tissue culture and for this reason give the impression that somaclonal variation "creates" new genes.

Evidence is already accumulating that transposable element activity is greatly enhanced during tissue culture. In maize, activity of the Activator component of the classical Ac-Ds transposable element system is greatly enhanced in plants regenerated from maize tissue culture (Peschke *et al.*, 1987). In alfalfa, the occurrence of an unstable anthocyanin mutation in plants regenerated from tissue culture has also been reported (Bingham and McCoy, 1986).

If enhanced transposable element activity is a consequence of tissue culture, then several opportunities exist for seemingly "new" genes to appear. Transposition of DNA sequences can relieve genes from preexisting repression of activity, or excision of an element from a structural gene can restore function to the gene product. Transpositions can also move structural gene sequences into regions where they fall under the control of different promoters, which could conceivably alter the level and developmental timing of gene expression.

A somaclonal variant has recently been described which affects the isozyme pattern of one of the seed protein complexes of wheat, namely, β-amylase (Ryan and Scowcroft, 1987). The mutant phenotype appears to be entirely new. This variant was found among 149 regenerants and was characterized by at least five new isozyme bands, as well as an increased intensity of two previously existing bands. This variant segregated with Mendelian fidelity. Recombination has not been observed between the variant bands, and mitosis and meiosis are cytologically normal both in the homozygous variant and in the F_1 between the variant and its parent. The variant pattern has not been observed in a survey of more than 100 diverse wheat genotypes that represent the 10 known β-amylase phenotypic groups. This new variant has not previously been observed and therefore gives the appearance of being a new gene. It could represent a rare mutation leading to the expression of a previously silenced locus.

Integration into Plant Improvement Programs

Controversy still surrounds the potential value of somaclonal variation in plant improvement. It should be remembered that mutants arising from cell culture are random and more likely than not will have deleterious effects. Like any mutation program, the likely value of any new mutant will depend on the objectives of the breeding program and the skill of the scientist to recognize them as useful.

a) *Agronomic Traits*

Several analyses have evaluated progeny populations of somaclones for variation in agronomic traits. Ryan *et al.* (1987) evaluated a total of 256 selected lines of wheat derived from somaclones of three different cultivars. Under replicated hill plot conditions, significant variation was found for all the characters evaluated, namely height, grain number per spike, kernel weight, yield, total dry weight, and harvest index. Subsequently, 32 lines selected from the original hill plot experiment were evaluated in larger replicated plots. None of the lines had superior yield performance or better harvest index relative to the parental lines. However, significant improvement in kernel weight, hardness, and protein content were found, and some lines had a significant reduction in yellow pigmentation of the grain.

b) *Sexually Reproducing Crop Species*

It is doubtful whether somaclonal variation can contribute a great deal of useful genetic variability to breeding in normal sexually reproducing species other than that which derives from the use of *in vitro* selection. *In vitro* selection is an efficient way to recover mutants within a particular cultivar that are tolerant to particular antimetabolites such as host-specific pathotoxins, some herbicides, and heavy metals. In these cases, somaclonal variation generates an array of mutants, and *in vitro* selection is utilized to enrich for those tolerant mutants of choice. Other deleterious mutants will also occur, but these can be rapidly eliminated by routine backcrossing.

c) Asexually Reproducing Species

For many asexually reproducing crop species such as sugarcane, pineapple, root crops, bananas, date palm, coconut, and numerous fruit trees, breeding through hybridization and recombination is difficult or impossible or very long term.

Coincidentally, many of these species are amenable to tissue culture and plant regeneration and, hence, somaclonal variation could have a significant role as a breeding tool. Frequently, cultivars of asexually propagated species are genotypically unique and an agricultural system has evolved around such cultivars. Therefore, genetic improvement of single traits affecting agronomic performance or quality features without altering the broad characteristics of the cultivar is highly desirable. In this case, somaclonal variation should be viewed as a means of generating potentially useful variability.

d) *Alien Gene Transfer*

One of the major underlying causes of somaclonal variation results from chromosomal rearrangement, i.e., translocations, insertions, etc. Frequently, alien gene introgression by interspecific hybridization is utilized in plant improvement, but introgression may be difficult because of the lack of homology between the genome of the crop species and that of the alien gene parent. Introgression as a consequence of mitotic exchange during tissue culture, i.e., somaclonal variation, is a valid option. Other unwanted exchanges will also occur but these can be eliminated by recurrent backcrossing. The use of somaclonal variation to enhance alien gene transfer requires a definite focus on breeding objectives and well-defined selection protocols for the target gene.

IARCs and the Private Sector—Strengths and Collaboration

Apart from addressing scientific issues, a major objective of this workshop is to identify areas of potential collaboration between the International Agricultural Research Centers and components of the private sector biotechnology industry.

The analysis of the various facets of a plant breeding and variety development program reveals that the IARCs and agricultural biotechnology companies have complementary strength. Collaborative relationships not only need to address the practicalities of introducing biotechnology into plant breeding but also downstream issues concerned with regulatory approval and protection of intellectual property.

A first positive step is to identify the respective strengths of the IARCs and the private sector. In this way, opportunities may be identified from the respective complementary strengths. Table 1

reflects the author's opinion on the respective abilities of the IARCs and the private sector in prosecuting what can be referred to as the Plant Breeding Cascade.

Table 1. Relative Strengths of IARCs and the Private Sector in Various Aspects of Plant Breeding.

	IARCs Sector	Private Sector
PREBREEDING		
• Producer needs	X	
• Market opportunities		X
• Germplasm acquisition	X	
• Biotech input		X
BREEDING AND VARIETY DEVELOPMENT		
• Hybridization and selection	X	
• Efficient screening		X
• Generation turnaround	X	
• Greenhouse, field evaluation	X	
• Enhanced germplasm	X	
• New gene systems		X
• Agronomic packaging	X	
• Processor evaluation		X
VARIETY EVALUATION, PRODUCT APPROVAL AND RELEASE		
• National evaluation	X	X
• International assessment	X	
• Regulatory approval		
— GE release		X
— food production approval		X
• Plant variety protection		X
• Seed increase and distribution	X	X

This cascade has three main components. The first phase is prebreeding which establishes the need for a plant breeding program either with a new crop or to refocus or enhance objectives toward a particular product or agronomic end point. In general, the IARCs are better skilled at identifying producer needs, particularly for less-developed countries, and accessing and assembling germplasm resources. The private sector understands how to address market opportunities and has been more aggressive in developing and adopting new and faster breeding technologies from plant biotechnology.

The main phase in the cascade is breeding and variety development. The IARCs and the private sector each have superior strengths in different aspects of the breeding program. The IARCs have a long and creditable record in hybridization and selection, enhancement of germplasm, evaluation of breeding lines, and developing the appropriate agronomy for the improved material. The private sector, on the other hand, tends to exploit efficient screening systems more effectively, readily develops new technology to isolate and introduce new gene systems into breeding programs, and places attention on downstream value of crop products through processor evaluation. Both the IARCs and the private sector seek to speed up generation turnaround in a breeding program.

The final phase of the breeding cascade is variety evaluation, product approval, and release. Both IARCs and the private sector are concerned with evaluation at the national level, but the private sector tends to be more concerned with evaluation for use by developed country agriculture, whereas the IARCs focus on the needs of less-developed and developing country national programs. Clearly, the IARCs are also far better equipped for international assessment. The private sector companies are already addressing the issues associated with regulatory approval of varieties developed with the input of genetic engineering. Similarly, new regulations governing approval of food products for human consumption put a new complexion on plant breeding enterprises. Not only has the private sector taken the lead in these areas, but government regulatory bodies appear to have singled out the private sector to establish legal precedent and case law. This issue is of concern primarily in developed western countries where seldom has public sector research taken the lead in active promotion of the benefits to be derived from the release of genetically engineered organisms. The developed countries are the test case for the use of new technologies to enhance plant variety development. The results of this, be it favorable or otherwise, will ultimately impact on plant variety development in those areas of the world serviced by the IARCs.

Protection of plant varieties under plant patent law is now available in some 18 countries and the number is expected to increase. Any collaborative effort between the IARCs and the private sector will need to address this issue perhaps as early as the prebreeding phase. For many of the agricultural biotechnology companies involved in plant breeding, the marketing and/or licensing of new varieties is a profit center. Therefore, the protection of intellectual property in the form of plant variety rights is essential for the private sector.

Finally, seed increase, certification, and distribution is of vital concern to both the private sector and the IARCs. Significant opportunities exist for collaboration in the more efficient production and distribution of seed, particularly hybrids, in both developing and developed countries.

The manner in which the IARCs and the private sector collaborate will have to be determined on a case-by-case basis. Negotiation of a workable and profitable relationship will be accelerated by an early recognition of the complementary strengths of the respective partners.

References

Bingham, E.T. and McCoy, T.J. 1986. Somaclonal variation in alfalfa. Plant Breed. Rev. 4:123-152.

Brettell, R.I.S., Dennis, E.S., Scowcroft, W.R., and Peacock, W.J. 1986a. Molecular analysis of a somaclonal mutant of maize alcohol dehydrogenase. Mol. Gen. Genet. 202:235-239.

Brettell, R.I.S., Pallotta, M.A., Gustafson, J.F., and Appels, R. 1986b. Variation at the Nor loci in triticale derived from tissue culture. Theor. Appl. Genet. 71:637-643.

Davies, P.A., Pallotta, M.A., Ryan, S.A., Scowcroft, W.R., and Larkin, P.J. 1986. Somaclonal variation in wheat: Genetic and cytogenetic characterization of alcohol dehydrogenase 1 mutants. Theor. Appl. Genet. 72:644-653.

Donn, G., Tischer, E., Smith, J.A., and Goodman, H. 1984. Herbicide resistant alfalfa cells: An example of gene amplification in plants. J. Mol. Appl. Genet. 2:621-635.

Evans, D.A. and Sharp, W.R. 1986. Applications of somaclonal variation. Biotechnol. 4:528-532.

Groose, R.W. and Bingham, E.T. 1986. An unstable anthocyanin mutation recovered from tissue culture of alfalfa (Medicago sativa). Plant Cell Rep. 5:104-107.

Karp, A. and Maddock, S.E. 1984. Chromosome variation in wheat plants regenerated from cultured immature embryos. Theor. Appl. Genet. 67:249-255.

Larkin, P.J. 1987. Somaclonal variation: History, method and meaning. Iowa State J. Res. 61:393-434.

Larkin, P.J. and Scowcroft, W.R. 1981. Somaclonal variation—A novel source of variability from cell culture for plant improvement. Theor. Appl. Genet. 60:197-214.

Larkin, P.J., Ryan, S.A., Brettell, R.I.S., and Scowcroft, W.R. 1984. Heritable somaclonal variation in wheat. Theor. Appl. Genet. 67:443-455.

Peerbolte, R., Ruigrok, P., Wullems, G., and Schilperoort, R. 1987. T-DNA rearrangements due to tissue culture: Somaclonal variation in crown gall tissues. Plant Mol. Biol. 9:51-57.

Peschke, V.M., Phillips, R.L., and Gengenbach, B.G. 1987. Discovery of transposable element activity among progeny of tissue culture derived plants. Science 238:804-807.

Ryan, S.A. and Scowcroft, W.R. 1987. A somaclonal variant of wheat with additional β-amylase isozymes. Theor. Appl. Genet. 73:459-464. Ryan, S.A., Larkin, P.J., and Ellison, F.W. 1987. Somaclonal variation in some agronomic and quality parameters in wheat. Theor. Appl. Genet. 74:77-82.

Scowcroft, W.R. 1985. Somaclonal variation: The myth of clonal uniformity. In: Hohn B, Dennis ES (eds.) Genetic flux in plants. Springer-Verlag, Berlin, p. 217.

Commercialization and Research Perspectives for Vaccines

Charles C. Muscoplat
Molecular Genetics, Inc.
Minnetonka, MN

Introduction

With the discovery in 1953 of the molecular structure of DNA came the birth of genetic engineering, a field whose most recent innovations have included such biotechnological developments as that of recombinant DNA technology which permits the altering of heredity by transplanting genes from one organism to another. Such an advance has made possible the manipulation of genetic materials in order to develop commercial products and processes. Further advances in immunology have been responsible for the development of hybridoma technology in which specific antibody molecules, termed monoclonal antibodies, have been responsible for additional advances in biotechnology. The impact of these technologies has been far-reaching with the most rapid developments occurring in agriculture, specifically in improved animal production. A variety of approaches have been developed including: 1. reducing animal losses by preventing infectious disease through genetically engineered vaccines and antitoxins; 2. increasing production of beef protein and milk through the use of growth promoters; and 3. improving the nutritional value of animal feed.

Biotechnology has promised a revolution in the development of animal health care products. Products envisioned include growth hormones to improve efficiency of livestock production and vaccines and monoclonal antibodies to treat and/or prevent infectious diseases. Beginning in 1980, many laboratories around the world began ambitious programs to genetically engineer subunit vaccines and prepare monoclonal antibodies. Numerous attempts at subunit vaccines against most major classes of viruses were difficult if not impossible. Reasons for this apparent failure are as yet unclear and may remain so for some time to come. Alternatives to subunit vaccines, which are being attempted by many laboratories, are things such as: i) cloning specific genes into vaccinia virus vectors, ii) genetically altering native viruses by deletion of virulence genes, and iii) producing native protein subunit vaccines through the use of improved separation techniques, specifically through the use of monoclonal antibodies. However, there is a real need for new vaccines for diseases that are as yet not controlled by any existing vac-

cine or product. For these diseases, the need and the challenges are great.

Recombinant DNA

Recombinant DNA technology is not a single discipline in itself but rather a fusion of ideas and techniques from biochemistry, molecular biology, genetics, and organic chemistry. It involves the restructuring and editing of genetic information and the construction of microorganisms with new genetic information. The technology allows one to isolate genes from any source, from virus, bacteria, fungi, plants, or animals, to amplify isolated genes to unlimited quantities through fermentation, and finally to manipulate genes by mutating or rearranging their components for the development of hybrid gene products. Essential to this process is the identification and isolation of specialized enzymes, termed restriction endonucleases, which act as biological scissors cutting DNA into unique pieces and enabling the isolation of specific genes or gene fragments. The result is a piece of DNA with complementary cohesive or "sticky" ends that can be recombined with another piece of DNA that has been cut by the same enzyme.

The basic recombinant DNA experiment is depicted in Fig. 1. The essential ingredients in this technology include: (1) a DNA vector that can replicate in living cells after foreign DNA is inserted into it. (Such a vector generally consists of the chromosome of either a plasmid, an autonomously replicating DNA molecule found in bacteria and yeast, or a virus that can infect bacteria or higher organisms.); (2) a DNA fragment to be inserted into the vector; (3) a method of joining the insert DNA to the vector; (4) a method of introducing the joined molecules, or recombinants, into a host that can replicate them; and (5) a method of detecting those cells that carry the desired recombinant DNA molecule. Once the vector carrying the inserted foreign DNA molecule is placed into an organisms such as a bacterium or yeast, it will replicate to make many copies of itself and the foreign gene insert, thereby providing an unlimited supply of the gene of interest. In order for the foreign gene insert to be expressed into protein in the bacterial cell, certain specific features that are important to the bacterium's biosynthetic machinery must be available to the gene (Fig. 2). For example, appropriate recognition signals for both bacteria-mediated transcription (RNA production) and translation (protein production) must be present.

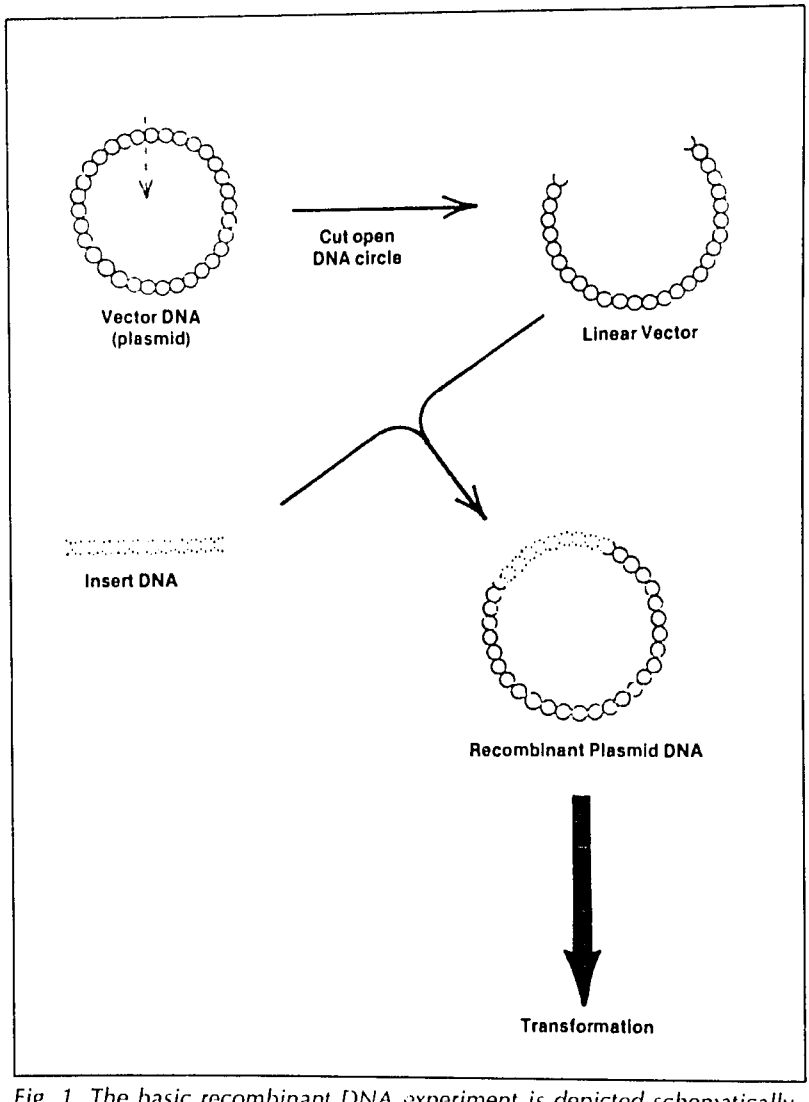


Fig. 1. The basic recombinant DNA experiment is depicted schematically.

Hybridoma Technology

Another major biotechnological development that will impact on animal health and production is hybridoma technology. This technique, which results in the generation of monoclonal antibodies by cell fusion procedures, will be useful for the diagnosis of specific diseases as well as for the therapeutic prevention and cure of diseases affecting the morbidity and mortality of farm animals. The procedure involves fusing spleen cells from mice immunized with an antigen to which a monoclonal antibody is desired to mouse

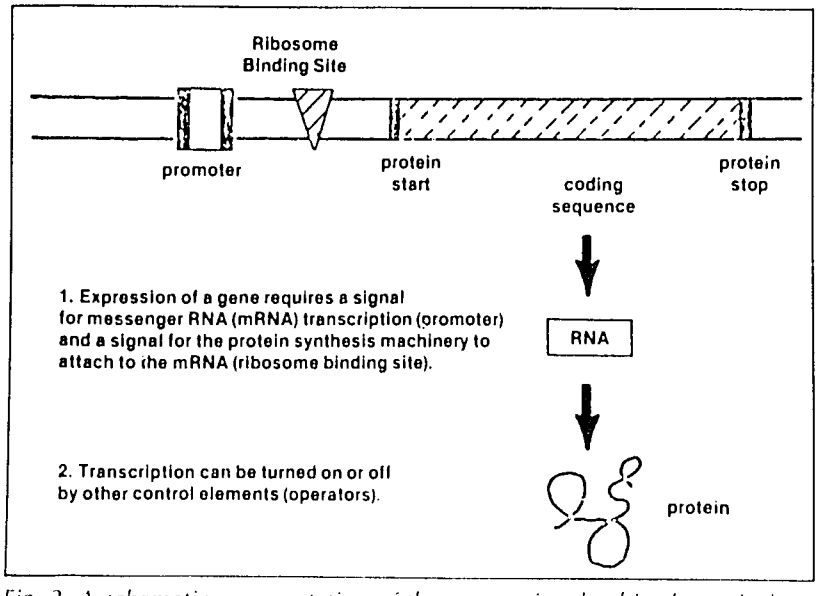


Fig. 2. A schematic representation of the process involved in the typical bacterial gene expression.

myeloma cells in culture and screening fused cells for production of the specific monoclonal antibody with the labeled antigen. The myeloma cells serve to immortalize the spleen cells so that they may be maintained indefinitely in cell culture. Specific procedures are employed such as the use of myeloma cells requiring certain growth factors provided by the spleen cells fused over unfused myeloma cells. The monoclonal antibodies can then be obtained by harvesting the liquid medium from the cell cultures or by injecting the fused cells into the peritoneum of mice and collecting the fluid present after ascites tumors have developed.

Vaccines and Antitoxins

One of the major ways in which recombinant DNA and hybridoma technologies will improve animal production will be to reduce morbidity and mortality from infectious disease. For example, since antibiotics are ineffective in reducing the severity of diseases caused by viruses, many virus-induced diseases go unchecked either because of the lack of an appropriate vaccine or because of the availability of an ineffective vaccine. Recombinant DNA procedures will enable the development of vaccines for infectious agents that grow poorly in cell culture. Moreover, a genetically engineered vaccine will be not only both potent and effective, but also safe, easy to manufacture, and economical to produce. It will have a long shelf-life, will lack infectious virus, and will be stable at am-

bient temperatures. Immunologically, the genetically engineered vaccine must be developed in such a way as to be administered in a single dose so as to induce immunity of long duration. It must protect against all serotypes in a given geographic region and must not induce adverse reactions. The protocol for developing a genetically engineered vaccine requires: (1) identification of the major surface antigen of the pathogenic organism of interest that will induce an antibody capable of neutralizing the infectious organism; (2) identification of the surface antigen gene or its specific antigenic determinants; and (3) isolation and transfer of this gene into a plasmic vector capable of expressing large amounts of its product in fermentable organisms such as bacteria or yeast (Fig. 3). Such methodologies have been employed to generate large amounts of vaccine proteins against bovine papillomavirus, porcine parvovirus, canine parvovirus, foot-and-mouth disease virus and K99 *E. coli*. Although reports of these vaccines are presently awaiting the completion of preclinical, clinical, and field trials, preliminary tests on several of these genetically engineered vaccines indicate that they are excellent immunogens.

Monoclonal antibodies have also been generated for protection of newborn calves and swine against enteric colibacillosos responsible for neonatal diarrhea or scours. Although both conventional and genetically engineered vaccines are available, the monoclonal antibody approach appears to be superior to vaccination for two reasons. First, vaccination of the dam requires anticipating the problem, which may not be feasible. Second, it requires that breeding records be maintained since vaccination must be given twice, at six and two weeks prior to birth. Since scours usually occurs within the first 24 hours of life, and since the action of the pathogenic bacteria is restricted to the intestine, oral administration of a protective monoclonal antibody to provide passive immunity within 12 hours of birth serves to protect newborns from developing the disease on farms where the disease is prevalent. In both preclinical and clinical testing to date (Table 1), a monoclonal antibody against the K99 strain of *E. coli* protected animals from lethal doses of the pathogenic strain of bacteria. The K99-specific monoclonal antibody appears to be group-specific, capable of reacting with the adhesive entity on the pilus of over 100 strains of *E. coli*. Additional attributes of this monoclonal antibody include reproducible specificity, high titer, and reduced costs. Similar monoclonal antibodies for two additional strains of pathogenic *E. coli* responsible for scours in newborn piglets (i.e., K88 and K987) have also been developed and are presently in preclinical testing.

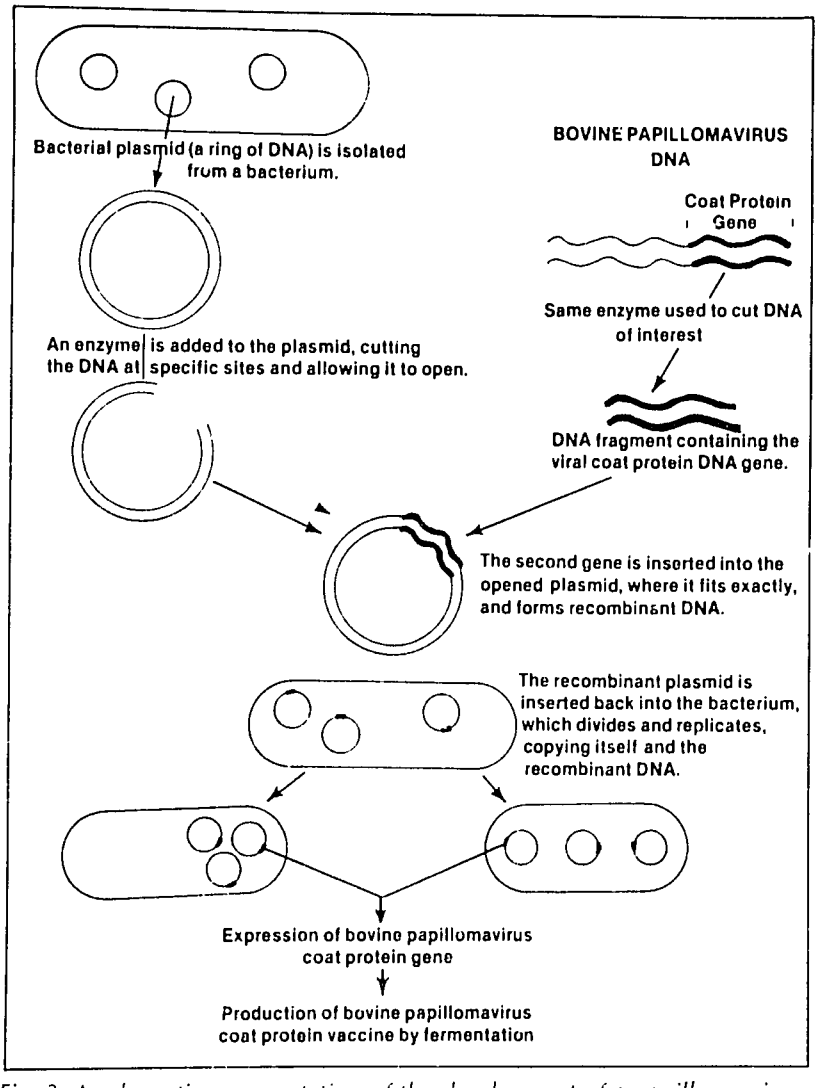


Fig. 3. A schematic representation of the development of a papilloma virus vaccine by recombinant DNA techniques.

Growth Promoters

The development of natural growth hormones for livestock and poultry represents a means of improving animal production, and genetic engineering techniques have made this development a reality. Several groups have now cloned bovine Growth Hormone (bGH) to expression in bacteria and yeast. The recombinant DNA procedures employed were similar to those utilized for cloning virus genes for vaccine production. A messenger RNA (mRNA) species from pituitary gland enriched for bovine growth hormone

Table 1. Protection of Newborn Pigs and Calves by the Oral Administration of a K99-Specific Monoclonal Antibody.

	Pigs		Calves	
Trial Group	Alive	Dead	Alive	Dead
Monoclonal antibody	11	3	9	1
Placebo	2	8	2	12

nucleotide sequences was first reverse-transcribed into DNA, and then this DNA copy was inserted into plasmids for expression in bacteria and yeast. Because this cow/bovine growth hormone gene lacks the required regulatory features necessary for these microorganisms to express this gene, some additional restructuring of the gene is required. One of the maneuvers results in the addition of an additional amino acid, methionine, at the beginning of the bovine growth hormone gene so it differs slightly from naturally occurring bovine growth hormones. Despite this additional amino acid, however, preliminary clinical studies have indicated that it is as effective as naturally occurring bGH in stimulating milk production. For instance, whereas milk yields were increased 10.3% for natural bGH over a six-day period of treatment, milk yields were increased by 12.9% for recombinant bGH. Neither milk fat, lactose nor protein percentages were affected by the treatment, and feed intake too remained unchanged. Feed efficiency (kg milk/kg feed) was improved by 9.5% and 15.2% for natural and recombinant bGH, respectively, and no adverse effects were observed based upon body temperature and somatic cell counts. Genetically engineered approaches to improving animal production, then, appear to be directly applicable where the availability of hormones and other natural growth promoters is limited and the costs prohibitive. Further studies are required to determine the safety of recombinant bGH for both treated animals and consumers of its milk. An additional growth promoter presently under development is porcine growth hormone.

Feed Improvement

Certain agricultural crops, particularly corn, are used as a major source of feed for animals. Corn is an excellent source of energy but a poor source of protein for hogs and poultry, and poor protein quality is directly related to deficiencies of essential amino acids such as lysine. Hog and poultry farmers must therefore purchase additional protein to supplement a corn-based ration if they are to

maximize such factors as rate of gain and feed efficiency. Recombinant DNA technology, however, offers an answer to this problem by providing a method for selecting specific desirable traits and excluding undesirable traits. In the case of corn, it is known that 50% of the bulk protein of the corn kernel is a storage protein referred to as zein. This zein storage protein is low in lysine and tryptophan, two essential amino acids for man and monogastric animals; consequently, these proteins influence the nutritional quality of the corn kernel. Recombinant DNA procedures have been utilized to isolate the zein gene and determine its precise biochemical structure. With the availability of this information, it is now possible to employ genetic-engineering approaches to alter the zein gene structure in an effort to increase its lysine content. Once this has been accomplished, the high-lysine zein gene can be transferred back into corn cells and corn plants regenerated with a higher lysine storage protein.

Although the former task is still in the experimental stages, the latter is not since tissue culture procedures, capable of plant regeneration initiated from juvenile tissues of corn, have already been developed. Simply stated, tissue culturing is the process whereby large populations of cells are stimulated by nutritional and hormonal conditions to grow in a defined laboratory environment. Shoot-meristems develop in large numbers in these cultures and under the proper conditions these meristems develop rapidly into complete plants that produce seed at maturity. In fact, the tissue-culture corn-regeneration technology has been useful in isolating amino acid-overproducing mutants by virtue of the fact that these tissues will randomly undergo spontaneous mutations in cell culture. Recently, overproducing mutants from the aspartate biosynthetic pathway, the pathway responsible for synthesis of lysine, threonine, methionine and isoleucine, were isolated due to an increase of approximately 100% in the level of threonine. This represents a 30 to 60% increase in the total threonine content of the kernel, an amount that would greatly improve the nutritional value of the grain if lysine and tryptophan were similarly increased.

Analysis of the threonine overproducers indicates that they are inherited as dominant mutations and have the advantage of creating very specific changes in the kernel such as selectively increasing the concentration of specific amino acids without causing unwanted pleiotropic effects in other kernel or plant characteristics. In a similar fashion, new strains of corn that offer resistance to disease, tolerance to herbicides, increased yields, and shorter maturation times will be developed as methodologies are improved.

Conclusion

It is evident that the industrialization of recombinant DNA technology can lead to useful products and processes. Because this is a basic methodology, the unforeseen applications may well be more important than any of those that have been proposed thus far. The underlying science of molecular biology and molecular genetics is dynamic, and it is reasonable to assume that new opportunities will be created as the depth of our scientific understanding increases. This new technology is surely no panacea. On the other hand, it has the potential of contributing significantly to the solution of some of the most difficult problems facing animal health and production today.

Vaccine Development: The Role of Biotechnology, ILRAD and the Private Sector

J.J. Doyle
The International Laboratory for Research on Animal Diseases
Nairobi, Kenya

Introduction

The International Laboratory for Research on Animal Diseases (ILRAD) is the Center of the Consultative Group for International Agricultural Research (CGIAR) that has a mandate to develop improved control measures for livestock diseases. The Laboratory was established in 1973, at a time when developments in immunology gave hope that new vaccines could be devised to control economically-important parasitic diseases of livestock of the developing world, especially Africa. This paper describes ILRAD's steps, since 1976, in developing one such vaccine against the tick-transmitted protozoan parasite *Theileria parva*, the causative agent of East Coast Fever (ECF) in cattle in East and Southern Africa. It is estimated that 25 million cattle in the region are at risk from this disease, which is currently controlled through acaricide application to kill the tick vector.

The Research Strategy

It was known, prior to the establishment of ILRAD, that the small numbers of cattle that recovered from the usually fatal disease, ECF, were resistant to further infection if they were maintained in the areas where they were infected. Movement to other areas generally resulted in further deaths. This observation led to the development of a vaccine based on living parasites obtained from infected ticks, administered at appropriate dilution with a dose of tetracycline antibiotic sufficient to prevent generalized infection. This vaccine was found to be parasite strain specific and a number of different strains needed to be incorporated into the vaccine to give broad protection in a region.

While effective, this vaccine is costly to produce, requires a cold chain for administration, and immunized animals remain carriers of the parasite. It was recognised that improved vaccines were necessary to provide sustained control of this disease and that ILRAD should devise such vaccines based on parasite components, rather than viable parasites.

Theileria parasites have complex life cycles in both ruminants and the tick vectors. Two stages of this life cycle were chosen as potential sites for immunological attack. The first is the sporozoite stage, the form injected by the infected tick, and the second is the schizont stage which results when sporozoites invade bovine lymphoid cells. The latter is the pathogenic stage of the parasite's life cycle in the bovine.

The research program has three major objectives. The first is to determine the nature of the protective bovine immune responses to both the sporozoite and schizont stages of the parasite's life cycle. The second is to identify and characterize the parasite antigens that induce protective immune responses. The third is to develop effective vaccines based on such parasite antigens.

The Development Strategy

As soon as it was shown that immunity to theileriosis was directed both at the sporozoite and schizont stages of the parasite, the questions of eventual vaccine production were considered. It was clear from the outset that molecular technologies would have a significant role to play in the development of novel vaccines against theileriosis for the following reasons: a) the sporozoite stage of the parasite cannot be obtained in sufficient quantities from infected ticks, or by in vitro culture, to provide sufficient materials for a widely applicable vaccine, b) while schizont-infected bovine lymphoid cells can be grown in culture, the costs of such culture systems are high and the techniques for obtaining purified parasite material too complex for routine vaccine production.

The implications of the use of molecular technologies were considered by ILRAD's Board and management and it was accepted that ILRAD itself could not, with its resources, carry out all the steps required to develop, test, and produce a novel vaccine. While ILRAD had a comparative advantage in identifying the antigens and immune mechanisms involved, the economic production of sufficient quantities of antigens to test their vaccine potential was not considered likely to be within ILRAD's capacity. The industrial sector was considered to be an important participant in the production of antigens by molecular technologies for vaccine testing. Industry has a clear comparative advantage over ILRAD in production technologies.

Patent Policy Development

In the light of this decision, the Board commissioned a review of its patent policy, by consultants experienced in this area, who met with interested parties in the public and private sectors before making their recommendations. The consultants reaffirmed the need for ILRAD to have a suitable policy on patents and related matters and the ILRAD Board of Directors redefined the policy, in 1984, as follows:

The Board of Directors of ILRAD, wishes to ensure that the findings accruing to the programme of research of ILRAD, and of such other programmes as ILRAD may be associated with as a grantor, grantee or collaborator, shall be of maximum public benefit. In furtherance to this intent, the Board has established the policy to retain for itself the proprietary rights to such discoveries, including the right to determine that the discovery shall be yielded into the public domain through disclosure, or to take out any copyright or patent in such work which enhances the possibility of achieving ILRAD's mandate either directly, or through provision of additional financial resources. In furtherance of this policy, all persons whose work is financed in part or whole by funds under the jurisdiction of the Board shall, as a condition of receiving such support, waive rights to such findings without the prior requirement that such be determined to be patentable by affixing their signature to one of the following agreements, whichever shall be most applicable.

1. *It is agreed that findings accruing to research funded in whole or inpart by ILRAD shall be utilised to maximum public benefit. Such research findings shall therefore be notified in writing to the Director General of ILRAD. ILRAD shall share property rights in the subject matter of such findings in the proportion equivalent to the amount which ILRAD investment bears to the total effort. This amount shall be determined by mutual agreement between ILRAD and the collaborator. In the event of conflict, the determination shall be made by a mutually agreed expert in the subject area.*

2. *Any data, research findings, information or discoveries made by any person whose partial or total salary is paid by ILRAD and accruing in any way from employment by ILRAD or use of facilities or funds made available by ILRAD become the sole property of ILRAD.*

ILRAD will therefore hold the initial patent on any discovery likely to be involved in obtaining new vaccines, i.e. the definite description of a protective antigen and its uses as an immunizing agent.

This will give ILRAD the freedom to determine how the vaccine would be subsequently derived in partnership with industry and applied to the benefit of the countries affected by ECF. Subsequent patents relating to development of the vaccine could be filed jointly by ILRAD and its commercial partner on grounds that are mutually agreeable. Prior to the filing of the master and subsequent patents, ILRAD's discussions with commercial organizations are to be held under confidentiality agreements agreed to by both parties. ILRAD has sought appropriate legal advice on the terms of such confidentiality agreements, before entering into them, so as to ensure ILRAD's interests were suitably protected.

Relationships with Industry

The next point to be considered by the Board and management was that, while ILRAD had considerable expertise in molecular biology, it did not have the facilities to carry out certain steps in the characterization of the parasite antigens and identification of their genes necessary to provide the relevant information for patent application. An approach was therefore made to a biotechnology company to carry out such work as a possible prelude to joint vaccine development. ILRAD discontinued this approach when it found that the costs proposed by the biotechnology company for carrying out the research were higher than those that would accrue to ILRAD if it increased its own research capacities and contracted certain aspects of the work, at cost, to nonprofit-making institutions.

This is the path that ILRAD followed and while the research program has possibly taken slightly longer to achieve its results, this policy has had the following advantages: a) ILRAD has maintained its proprietary rights, b) ILRAD gained much relevant expertise to apply to other research problems, and c) the research has been carried out in an economic manner and donor resources have been available for other research.

ILRAD has now identified, characterized and isolated the genes coding for two sporozoite antigens, one of which clearly induces the formation of protective antibodies in cattle. This antigen is present on the surface of sporozoites from different parasite strains and therefore represents a potential cross-strain vaccine candidate. Another three potential protective antigens are also currently being characterized. The research team, including those responsible for production of parasite material, currently involves four senior scientific man years/year and two senior technical staff man years/year. Amino-acid sequencing, oligonucleotide and peptide preparation were carried out in collaboration with the Wistar Institute, Philadelphia on a cost-recovery basis.

ILRAD has continued the search for a suitable industrial partner for production of parasite antigens and vaccine development, but has moved its attention from the newly formed biotechnology companies that, because of dependence on venture capital, are unlikely to make a commitment to a vaccine for parasitic disease in African livestock because of potentially low capital returns, to the established major pharmaceutical firms with both experience in molecular technologies and vaccine development. ILRAD has had fruitful discussions with a major American company whose subsidiary is the largest manufacturer of veterinary vaccines in the USA. The basis of the discussions has been that while the company has expertise and facilities for antigen production using recombinant DNA systems, and in vaccine production, ILRAD has expertise and facilities for parasite antigen identification and isolation, for testing of antigens and developing potential vaccines. In practical terms the agreement being developed is that ILRAD scientists will make use of the facilities and expertise of the company in recombinant DNA expression systems to produce sufficient quantities of sporozoite antigens for initial testing, at ILRAD, of their vaccine potential. If these results are encouraging then further discussions will be held with the company to reach agreement on the terms for vaccine development and eventual production.

Vaccine Development

This will be a joint exercise using the expertise of both parties. There are several options for vaccine design based either on the antigen itself, in combination with different chemical adjuvants, or on its gene, using the newly developing systems of viral vectors. An ideal vaccine would give life-long immunity following a single administration in all ages and breeds of cattle. It should be stable under a wide range of environmental conditions and simple to manufacture and monitor quality control. ILRAD has the necessary technology for monitoring the immune responses, both antibody and cell-mediated, induced by different types of vaccine and to relate these responses to the level of protection obtained. It also, by virtue of its work on the bovine immune system, may identify novel ways of antigen presentation to improve the efficacy of vaccines. The use of peptide sequences known to engage T helper-cells as adjuvants or the use of bovine interleukins as potential adjuvants are examples of new technologies that could be applied. Through collaboration with industry, ILRAD would also have access to vaccine technologies that it could not obtain directly from the public sector. The final choice of vaccine design will determine its production technology and hence both cost and complexity of manufacture.

Virally vectored vaccines theoretically would appear to have the required characteristics and their use will be carefully explored. There are, however, questions concerning the eventual licensing of such vaccines for use in the countries affected by ECF, that have to be resolved before any final decision is taken on vaccine design. Once constructed, however, virally vectored vaccines can be manufactured using technologies and equipment similar to those used for other viral vaccines such as foot and mouth disease. Such facilities are present in certain of the countries affected by ECF and could potentially be used for local production of vaccines against ECF under license from ILRAD and its collaborating pharmaceutical company. More conventional vaccines using recombinant DNA technologies for antigen production may be too complex to produce, at the present time, in most of the countries affected by ECF.

Conclusions

It has been ILRAD's experience that, in order to benefit from the new technologies to produce novel vaccines against ECF, a careful analysis of the areas of comparative advantage in the stages of research necessary to develop the vaccines helped greatly to identify the respective roles of ILRAD and the private sector. This led to the revision of ILRAD's policy on matters relating to patents, so as to ensure that ILRAD's research findings would ultimately lead to a vaccine that would be produced and applied to the benefit of the countries affected by ECF. The analysis also defined the characteristics and capacities required in the industrial partner that aided in the identification of suitable companies with which to discuss potential collaboration. In practice it has been found most useful to develop collaborative agreements that cover specific phases of the research process, rather than trying to initially put together an agreement that covers everything, up to and including vaccine production and distribution. It is important that both parties clearly understand the concerns of the other and that they keep faith with any agreements that are ultimately formulated.

The concept of using the technologies available in the private sector in the developed countries to benefit the agricultural sector of the less-developed countries is not new. Bilateral aid programs generally have a strong bias towards the products of the donor country. The concern, however, is to ensure that the benefits of modern technology are made available to such countries to solve previously intractable problems of agricultural production. ILRAD sees its role as an intermediary in this process where its scientific expertise can form the link between the industrial community of the developed world and the problems of the livestock owner in Africa.

Genetic Transformation for Virus Resistance: Needs and Opportunities in Developing Countries

Roger N. Beachy
Professor of Biology
Washington University
St. Louis, Missouri

The work that I will present results from interactions between scientists at Washington University and Monsanto Company and was built upon more than 50 years of basic science. Likewise, the application of the research technology described here to situations in developing countries will also require a team approach. In this paper I will not describe in detail the molecular biology that was done to produce genetically transformed plants that are virus resistant, but intend to convey the basic concepts and features of the procedure, emphasizing the simplicity of the research. The work was initiated at Washington University in 1981 and 1982, and collaboration with scientists at Monsanto was established soon thereafter. I'll primarily discuss the work in my laboratory, and then will include a short description of the work done at Monsanto, followed by a brief discussion of the field experiment with genetically engineered plants that was carried out in 1987, and lastly, the opportunities for collaboration that might impact agriculture in other countries.

There are, as you know, many examples of plant virus diseases. Our work began with tobacco mosaic virus (TMV), which causes a disease in tobacco as well as tomato and a number of other solanaceous plant crops. TMV has been well characterized during the last 50 years, and a great deal is known about its genetics, the biochemistry of the disease it causes, and the biophysics of virus assembly (van Regenmortel and Fraenkel-Conrat, 1986). The technical approach used to produce genetically transformed plants that harbor a viral gene has been described in other reports (Powell Abel *el al.*, 1986; Beachy *el al.*, 1987). In brief, we enzymatically produced a cDNA (copy DNA) from TMV-RNA that encoded the TMV coat protein, and attached the double-stranded cDNA regulatory elements to produce a chimeric gene. A transcriptional promoter was taken from another plant virus, cauliflower mosaic virus, to control the expression of the coat protein gene in transgenic plants. The chimeric gene was constructed in a plasmid obtained from Monsanto Company that contains many of the features which are needed for transformation of plant cells. This plasmid was mobilized into a disarmed strain of *Agrobacterium tumefaciens;* the modified *A. tumefaciens* was subsequently used

for plant transformation reactions. The plant transformation procedures are covered in greater detail in another chapter in this volume. After carrying out transformation and regeneration of transgenic plants, the transformed plants were identified on the basis of their genotype and phenotype.

The level of expression of the coat protein gene was determined in transgenic plants and those with high levels of gene expression were selected for further study. Seeds collected from the parent transformants were planted, and the segregation ratios of the inserted genes was determined by analysis of the seedlings. If there was an active gene at a single locus, one expected a 3:1 segregation of the introduced gene. The plants that expressed the introduced coat protein gene and those that lacked the gene were then inoculated with TMV, and disease development and virus replication were monitored. Plants that lacked the coat protein gene (i.e., the control plants) developed disease symptoms in 3 to 4 days, while plants that expressed the gene either escaped infection or developed symptoms much later (in time) than did the control plants (Powell Abel *et al.*, 1986). This type of resistance is referred to as "coat protein protection" or "genetically engineered cross-protection." We have extensively characterized transgenic tobacco and tomato plants that are resistant to TMV due to "coat protein protection." The features of the resistance included the following facts: (1) resistance is largely overcome by inoculation with high levels of TMV (Powell Abel *et al.*, 1986), or by inoculation with TMV-RNA (Nelson *et al.*, 1987); (2) plants that express the TMV coat protein gene are resistant to several strains of TMV (Nelson *et al.*, 1987) and to several strains of tomato mosaic virus (Nelson *et al.*, 1988); (3) plants that express the TMV coat protein gene are not highly resistant to infection by other viruses (E. Anderson and R.N. Beachy, manuscript in preparation); and (4) the level of resistance to infection is directly related to the level of expression of the gene and the accumulation of coat protein.

Since there are many different kinds of plant viruses, a question is raised as to whether this approach will provide protection only against tobacco mosaic virus and other tobamoviruses, or for many different kinds of plant viruses. The answer is that the approach is successful with many different kinds of viruses. Resistance against tobacco rattle virus, a rod-shaped virus whose genome is split into two particles, was recently reported by van Dun and Bol (submitted). Several groups have produced resistance against alfalfa mosaic virus, a virus whose genome is divided into three bacilliform-shaped particles (Tumer *et al.*, 1987; Loesch-Fries *et al.*, 1987; van Dun *et al.*, 1987). Protection against a flexuous, rod-shaped virus, potato virus X, was developed through collaboration

between researchers at Monsanto Company and Rockefeller University (Hemenway *et al.*, 1988). These groups also collaborated to produce resistance against cucumber mosaic virus, an icosahedral virus with a genome that is divided in three parts (Cuozzo *et al.*, 1987).

The conclusion thus far is that even though the virus particle shape and the replication strategies (i.e., the mechanisms of virus replication and gene expression) are different with each of these viruses, expression of the capsid protein gene in transgenic plants provides resistance against the virus from which the gene was isolated. It is therefore proposed that this represents a generic method to produce virus resistance. Furthermore, the method may provide virus-resistant material that can be given to the plant breeder for introgression of the gene into his own breeding stock. In essence the pathogen provides a novel source for a disease-resistance gene, while transformation provides a means for introducing the gene into agronomically desirable cultivars. This approach has the potential to enormously simplify the work of plant breeders who generally search for resistance genes in the other plant varieties, cultivars, or species, and spend years improving the agronomic characteristics by a backcrossing regimen.

Evaluating the degree of disease resistance is an important concern for the plant pathologist. We found that the most direct way to assess resistance to virus infection was to inoculate populations of susceptible plants and transformed plants with increasing concentrations of TMV. When the control plants were inoculated with TMV at 0.001 $\mu g/ml$, 55% of the plants showed disease symptoms within 5 days. When the inoculum was increased tenfold, all of the control plants showed disease symptoms within 5 days. On the other hand, the transgenic plants that expressed the TMV coat protein gene required 10^4 higher levels of inoculum to become diseased in 5 days. This is referred to as 4-orders of magnitude of resistance, and provides a level of protection to withstand high levels of disease potential. The reader is reminded that this degree of resistance was accomplished in one generation of transformation, and only a relatively short period of time was required to produce resistance to TMV in tobacco and tomato plants.

Toward an Understanding of Engineered Disease Resistance

What is the basis of engineered coat protein protection? Although a full understanding of engineered protection is not yet known, it apparently is not like other types of genetic resistance that have, thus far, been studied and can be referred to as a type of nonhost resistance. In engineered protection, most cells don't become in-

fected upon inoculation. If a few cells become infected, the virus moves very slowly, if at all, from the inoculated leaves throughout the plant, indicating that there are multiple stages or manifestations of resistance.

The first evidence of the protection is the resistance to infection upon inoculation. Infection events can be assessed by counting the number of sites of infection that are visible as chlorotic or necrotic lesions (Nelson *et al.*, 1987), as percentage of infected protoplasts (Register *et al.*, 1988), or by counting starch lesions (Hemenway *et al.*, 1988) following inoculation. Resistance to inoculation with TMV is reflected as an 85-95% reduction in the numbers of sites of infection on leaves of protected plants compared to control plants (Nelson et al., 1987). In many cases, the plant would not receive sufficient levels of inoculant to produce infection and thus escapes infection.

The second manifestation of resistance is expressed even if a site of infection is established; the virus is unable or is less likely to move from the infected leaf to other parts of the plant. Disease requires infection, replication, and spread of virus through the vascular system to the upper leaves where it may cause the development of symptoms on the newly developed young leaves. Since the spread of virus is essential to disease development, we began to look at the rate of virus movement from one leaf to the next in transgenic plants compared to nontransgenic plants. For these studies we inoculated the plants with TMV-RNA, which largely overcomes resistance (Nelson *et al.*, 1987). When the movement of virus to the upper leaves was followed, it was found that systemic infection occurred in some, but not in all of the plants. Equally as remarkable is the observation that in some plants equal amounts of TMV accumulated in the inoculated leaves of both the control and coat protein expressing plants. Yet the virus does not move systemically (Powell, Nelson, Wisniewski and Beachy, work in progress). We are continuing our investigation into the nature of the blockage of systematic spread of virus in the transgenic plants that express the TMV coat protein gene.

The First Field Test of Transgenic Plants for Virus Resistance

In 1987, we collaborated with scientists at the Monsanto Company in an experimental field trial of the transgenic tomato plants. This was done only after full compliance with guidelines established by governmental regulatory agencies. Tomato plants were grown in a field situated in West Central Illinois and were inoculated with TMV by hand inoculation. The experimental plot design was a randomized split plot. Border rows which were not inoculated were

used as control plants for visual observations. At early stages in the experiment one could readily distinguish differences in growth of the inoculated control plants and transgenic plants. As anticipated from results of earlier glasshouse experiments, the inoculated plants that did not express the coat protein gene (i.e., the control plants) became diseased in the expected period of time, while only 8-10% of the plants that expressed the coat protein gene became infected and developed disease symptoms (Nelson *et al.*, 1988).

The yields of tomato fruits were also determined in the field experiments. In the two lines of plants that were not inoculated the yields were the same, indicating that the expression of the foreign gene (i.e., the coat protein gene) had no effect on plant growth, development, or yield. This corroborates the results of our earlier glasshouse experiments. However, infection with TMV caused a 25-30% loss in yield in control plants, whereas inoculation of the transformed plants led to little infection and, consequently, no reduction of yield (Nelson *et al.*, 1988). These results conclusively demonstrated the degree of protection against infection and yield loss as the result of expression of the coat protein gene.

Application of Coat Protein Protection to Agricultural Problems in Developing Countries

I recently had the privilege of visiting several Asian countries where there are opportunities to observe many disease problems that are caused by different plant viruses. The virus diseases that occur throughout Asia severely reduce the yields of most of the vegetable and fruit crops; for example, papaya orchards in which 80-90% of the plants were infected with papaya ringspot were common. Virus diseases in melons and other curcurbits were also severe and caused losses in fruit size and quality. Many of the affected crops are dicotyledonous and are related to crops used in plant transformation and regeneration experiments in laboratories around the world; therefore, they are reasonable targets for genetically engineered cross-protection. Although there are numerous target crops for application of this technology in developing countries, establishing research programs to achieve the targets will require international collaboration because of the degree of technical sophistication involved. It is unlikely that such research will be possible in many countries simply because of unreliable power supplies, the cost of equipment and chemicals needed for the research, or the unavailability of isotopes and perishable reagents. On the other hand, selected university laboratories and international research centers will be capable of carrying out the work with the arrival of appropriately trained scientists, and in collaboration with scientists in developed countries.

However, even in such special situations there will most likely need to be extensive financial and technical input from developed countries if recombinant DNA technology is to have an impact to improve the quality of food production in less-developed countries. This will include the need for improved facilities, education, and technical training of scientists, in addition to making certain that a ready source of specialized reagents and other supplies is in place. In some cases, this will involve problems associated with foreign currency exchange, and other issues that may hinder scientific research. Collaborative arrangements with other scientists who can provide supplies or financial resources will be essential.

It will also be necessary to deal with issues that involve patents and intellectual property rights since for much of the specialized technology in the field of recombinant DNA research is either patented or patents are pending. Although this, too, like the currency issue, can be complex and a hindrance to scientific progress, the prospects are sufficiently important that this issue must be dealt with in a manner that recognizes the investment of those who developed the technologies, yet considers the needs of the user countries.

Finally, one must be prepared to expend the necessary effort to satisfy the concerns of the public regarding the relative safety and possible risks in the use of transgenic plants in agricultural settings. Although there is an air of optimism in the scientific community that all or most objections can be satisfied, it is essential that each planned release and use be carefully evaluated by knowledgeable scientists familiar with each test site to allay any fear or misgiving of less-knowledgeable members of the lay public.

Because we are confident that genetically engineered cross-protection can play an important role in developing countries, we (R.N.B. and Dr. Claude Fauquet, Ph.D., ORSTOM, 213 Rue Lafayette, Paris, France; current address: as for R.N.B.) will cooperate with several donor agencies to establish a research program to alter agronomic characteristics of cassava by genetic transformation. This program, THE INTERNATIONAL CASSAVA TRANS PROGRAM, will have as its primary goal the transformation and regeneration of cassava, targeting resistance to virus diseases in the first 2 to 3 years of the program. It is anticipated that scientists from Europe, the United States, Africa, Asia, and South America will participate in the program. At the time of this writing, it is anticipated that funding will be provided by French, German, and English governmental sources, and the Rockefeller Foundation. Furthermore, commitment to collaborate in the project has been made by scientists and administrators at the two major cassava

research and germplasm collection centers, i.e., CIAT in Colombia and IITA in Kenya. Furthermore, the Monsanto Company, a leader in the use of biotechnology in agriculture, has agreed to lead technical support and consultation for the research. CASSAVA TRANS will be established in the Department of Biology at Washington University, St. Louis, Missouri, during Phase I, which will determine the technical feasibility of the project. It is anticipated that the project will later move to a European location for continued long-term research and interaction with scientific research institutions in developing countries.

The costs of establishing and maintaining CASSAVA TRANS are not trivial, and a great deal of collaboration will be required to bring it success. Nevertheless, we are committed to it as an example of the application of biotechnology to agriculture in the developing countries.

(The authors welcome inquiries about CASSAVA TRANS from possible donors as well as scientists interested in participating in the project.)

Acknowledgments

Ms. Nancy Burkhart is thanked and commended for her patience as well as skill during the preparation of the manuscript. I also acknowledge the support from the Monsanto Company and the National Science Foundation for the work described here.

References

Beachy, R.N., D.M. Stark, C.M. Deom, M.J. Oliver, and R.T. Fraley. 1987. Expression of sequences of tobacco mosaic virus in transgenic plants and their role in disease resistance. *In:* "Tailoring Genes for Crop Improvement," eds. G. Bruening, J. Harada, T. Kosuge, and J. Hallaender, Plenum Publ. Corp., N.Y., pp. 169-180.

Cuozzo, M., K.M. O'Connell, W. Kaniewski, R.-X. Fang, N.-H. Chua, and N.W. Tumer. 1988. Viral protection in transgenic plants expressing the cucumber mosaic virus coat protein or its antisense RNA. Bio/Technology 6:549-557.

Hemenway, C., R.-X. Fang, W.K. Kaniewski, N.-H. Chua, and N.E. Tumer. 1988. Analysis of the mechanism of protection in transgenic plants expressing the potato virus X coat protein or its antisense RNA. The EMBO. J. 7:1273-1280.

Loesch-Fries, L.S., D. Merlo, T. Zinnen, L. Burhop, K. Hill, K. Drahn, N. Jarvis, S. Nelson, and E. Halk. 1987. Expression of alfalfa mosaic virus RNA 4 in transgenic plants confers resistance. EMBO J. 6:1845-1852.

Nelson, R.S., P. Powell Abel, and R.N. Beachy. 1987. Lesions and virus accumulation in inoculated transgenic tobacco plants expressing the coat protein gene of tobacco mosaic virus. Virology 158:126-132.

Nelson, R.S., S.M. McCormick, X. Delannay, P. Dubé, J. Layton, E.J. Anderson, M. Kaniewska, R.K. Proksch, R.B. Horsch, S.G. Rogers, R.T. Fraley, and R.N. Beachy. 1988. Virus tolerance, plant growth, and field performance of transgenic tomato plants expressing coat protein from tobacco mosaic virus. Bio/Technology 6:403-409.

Powell Abel, P.A., R.S. Nelson, Baron De, N. Hoffmann, S.G. Rogers, R.T. Fraley, and R.N. Beachy. 1986. Delay of disease development in transgenic plants that express the tobacco mosaic virus coat protein gene. Science 232:738-743.

Register, J.C. III and R.N. Beachy. 1988. Resistance to TMV in transgenic plants results from interference with an early event in infection. Virology (in press).

Tumer, N.E., K.M. O'Connell, R.S. Nelson, P.R. Sanders, and R.N. Beachy. 1987. Expression of alfalfa mosaic virus coat protein gene confers cross-protection in transgenic tobacco and tomato plants. EMBO J. 6:1181-1188.

van Dun, C.M.D. and J.F. Bol. Transgenic tobacco plants accumulating tobacco rattle virus coat protein resist infection with tobacco rattle virus and pea early browning virus (submitted).

van Dun, C.M.D., J.F. Bol, and L. Van Vloten-Doting. 1987. Expression of alfalfa mosaic virus and tobacco rattle virus coat protein genes in transgenic tobacco plants. Virology 159:299-305.

van Regenmortel, M.H. and H. Fraenkel-Conrat, eds. 1986. The Plant Viruses, Vol. 2, The Rod-Shaped Plant Viruses. Plenum Press, N.Y.

Genetically Engineered Insect Resistance in Crops

Ronald Meeusen
Director, Plant Biotechnology Research
Zoecon Research Institute
Palo Alto, California

I confess I was very impressed to hear just now of the degree of collaboration that Dr. Beachy's group and others have managed to structure with international crop-breeding programs in the virus resistance area. Today, what I'd like to do is talk to you about some of the research that's coming out of the private sector along these lines, in this case dealing with insect resistance rather than with virus resistance, but using many similar techniques. And at the end of my talk, I'd like to turn from the status of the technology to the rather more difficult question of how nonprofit organizations like yours might interact with the private sector when technology like this originates there rather than from academe.

The Rohm and Haas Company is a chemical company based in Philadelphia and has been involved in agriculture since 1927, when they introduced one of the first totally synthetic organic insecticides. Since that time, as you are all well aware, agriculture in the United States and in the industrial countries as a whole has benefited dramatically from the inputs of both public and private sector research in the areas of agricultural chemicals, chemical fertilizers, mechanization, and crop breeding. Corn has been one of the most dramatic examples. Other crops—soybeans, wheat, you can name almost any major crop—show the same steady incremental improvements in the yield per acre.

A major component of these gains results from improved control of insect pests made possible by a continuous stream of new chemical insecticide products introduced since World War II. And these products, in turn, resulted from the coupling of new technology (in this case, advances in organic chemistry) to the development of a strong chemical patent system.

Thus, organizations in the industrialized countries found themselves with the ability to produce needed new products and the incentive to do so. The result over the past four decades has been a steady supply of chemical insecticides with increasingly greater environmental acceptability, human safety, and cost performance. New insecticides, introduced since the 1940s began with the so-called "miracle" insecticides, which were effective at some 2 to 4 kilograms per hectare. Today, that same job is being done with

products that are being recommended at rates in the 5 to 10 grams per hectare range. Both savings to growers and reduced effects on the environment follow.

This is an example of optimizing insect control through increased investment in research into traditional technologies like organic chemistry. But despite the progress already made, insect problems persist. In most major crops like cotton, the grower still spends a large proportion of his production outlays on chemicals for insect control. And the point I want to make is that seed is traditionally much cheaper than agrochemicals. You buy it once, you don't have to buy it eight or nine times throughout the season. In a pinch you can save the seed and plant it over a second year.

Even in the industrialized nations, insect control with these very effective chemicals is still a much more expensive operation than if one could control insects through use of a resistant seed variety. And that brings us to the research that I want to talk about today, an alternative approach to chemicals whose development has been sponsored by the chemical industry.

Now the Rohm and Haas Company and other agrochemical companies do currently sell chemical insecticides. But that is not really their business. Their business is providing products to the grower that protect his crops against insect pests. That's a subtle difference, but a very important one. From 1927 until today, companies like the Rohm and Haas Company invested their money in producing new and better chemicals, largely because that was the only effective technology available which enjoyed patent protection.

In the 1970s that began to change. Advances in molecular biology promised the ability to manipulate genetics with the same degree of efficiency, the same degree of precision that we had previously been able to apply to tailoring chemical structures and chemical properties. As these new tools became available, companies began applying them to their customers' needs, a logical extension for companies whose business is providing products, be they chemicals, seed or whatever, to protect crops.

And in the early 1980s, Rohm and Haas, Monsanto, and many other companies began to put some of their research dollars into biological-based research that would ultimately lead to crop varieties resistant to insect control and, as Dr. Beachy described, virus resistance.

That is not an insignificant shift, by the way. Last year, in the industrialized nations, the private sector pumped 850 million dollars

into research on agrochemicals. If even a small portion of that gets redirected towards biologically based systems, it can have a dramatic effect on the total amount of research and new products available to society.

So back in the early 80s, Rohm and Haas and other companies began looking to test the utility of these new techniques, to see if we could use them to actually produce something useful. One of the things we settled on was attempting to insert into crops a gene for a natural insecticidal material to see if we could not, in a sense, immunize the crops against insect predation in much the same way that Dr. Beachy has just described—immunizing crops against viral attack. And not surprisingly, we picked one of the best known materials, *Bacillus thuringiensis* (B.t.)with which I'm sure most of you are familiar.

B.t. has been used in the United States since the 1960s. It's very active against caterpillars of the order *Lepidoptera* and has become an environmental favorite due to its extraordinary selectivity, safety, and biodegradability. When the *B.t.* bacterium sporulates, it produces a protein that crystallizes outside the spore. Once ingested by a caterpillar, this protein crystal dissolves, the protein attacks the gut lining, gives the caterpillar ulcers, and stops the insect from feeding. Over a period of a couple of days the insect dies.

Since the isolated protein was known to be effective even without the bacterium, the idea was to take its single gene out of the bacterium, put it into a form where it would function in a plant and produce plants that now, in their leaves, produce minute quantities of this safe and biodegradable insecticide. If so, the resulting plants should be resistant to caterpillars.

Rohm and Haas went to a startup company in Belgium, Plant Genetic Systems, that had access to much of this technology, and set this as a research contract (not an uncommon thing for companies to do when they want to test the waters).

And I thought I'd take just a minute to describe to you the process that's used to get a gene into a plant, since it is an excellent example of applied biology in its own right. In order to get a gene into a crop plant, we use another bacterium, *Agrobacterium tumefaciens*, that causes the galls commonly seen on plants. This bacterium has a fascinating life cycle that we make use of.

When this bacterium infects a cut in a leaf, it will transfer to a single plant cell a piece of DNA, so it's a little genetic engineer all by itself. In the process it gives that plant cell genes that code for

two functions: one causes the cell to produce growth hormones, so that infected cells grow rapidly into a large clump. That gives you the gall that we can see on leaves and petioles in trees. The second set of genes that it gives this plant cell causes the plant to produce large quantities of unusual amino acids that the plant can't use, but that the bacterium can live on. So this bacterium actually engineers a small gall in the plant that produces food for the bacterium and allows it to multiply.

How does this unusual way of earning a living help us get genes into plants? Fortunately, bacteria are somewhat more easily duped than people. If we remove the normal gall genes, replace them with a gene we want in the plant, and then infect the plant with the bacterium, it will go blindly ahead and put in the gene we want, never noticing the difference.

One takes the crown gall bacterium, replaces the gall genes with the desired gene, in this case a gene for the *B.t.* insecticidal protein, and uses that bacterium to infect a piece of leaf tissue. Then one kills off the bacterium with antibiotics, leaving a leaf disk with a few cells around its edges that have now picked up the new genes. These are grown back into small clumps of tissue through tissue culture and eventually regenerate a whole plant. That whole process in tobacco is about a four-month cycle, and so you can go through three cycles of this in a year if you need to. Using that system then, we went after the *Bacillus thuringiensis* gene, which is active against caterpillars.

We are all familiar with this group of insect pests, the Lepidoptera. They comprise a hundred thousand species and constitute one of the most destructive groups of pests in the world. And virtually every crop has some Lepidopteran pest. That made it a particularly attractive test case. We had a single gene from a known material that is a registered pesticide, is known to be nontoxic to humans and wildlife, is known to be biodegradable, and is active against the class of caterpillars that are problems in many, many crops. It seemed like an ideal case on which to test out this technology.

For those who may never have had the dubious pleasure of seeing these pests firsthand, there is a good reason they are so destructive. A freshly hatched caterpillar, which weighs about 0.5 milligrams will grow to about 5 grams in 10 to 12 days. It does this by eating voraciously.

To shorten a long story, when we went to this process we did get tobacco plants that contained the gene, which produced less than .01 percent of their leaf protein as this new insecticidal protein. So

we are putting a tremendously small quantity or diverting a very small quantity of the plant's protein into this new protein.

Two plants each had 12 freshly hatched caterpillars put on their leaves. We shut the door, left them for two weeks, came back, and found that not only have all caterpillars attempting to feed on the new plant died, but that the newly hatched caterpillars do in fact feed on the transgenic plants. They begin feeding and punch about a two millimeter diameter hole. At that point they've eaten enough tissue to have received a lethal dose. They will often crawl to the edge of the leaf and die. Sometimes they will try to climb down the stem but they seldom get far. We get 100 percent control of these caterpillars.

If you look very closely at the leaves, you can often, especially on tobacco because the leaves are sticky, find the *corpus delicti* next to the scene of the crime. Having done this in the laboratory, of course we wanted to get out to the field. And that requires some traditional breeding work. We've now taken this, by the way, to the sixth generation. It breeds as a simple Mendelian dominant. We've seen no side effects in the plants and we've been able to cross it into six commercial tobacco varieties as well using traditional breeding.

In 1986 and 1987 we were ready to go to the field. And I could spend an hour of your time today talking about dealing with public perceptions and the federal regulatory complex. "Complex" is a good word, by the way. Xavier will talk about that later on. But suffice it to say that at the time we went to the government, we had no idea how they or the public would respond to this kind of work.

At the time we approached the U.S. Department of Agriculture the new regulations for such products were still being drafted. Although not legally required to seek approval, we naturally submitted a data package voluntarily, in effect offering our application as a test case on which the proposed new regulations could be tested.

Once we had been notified that approval was imminent, we decided to take one further step, a public communication campaign. We felt in this case, since we were dealing with something new and this would be one of the first field tests of a recombinant crop plant in the world, it was probably best to put together brochures, to send teams out to talk to the federal, state, and local officials, and eventually we ended up holding public seminars and media presentations prior to going ahead with the field test.

With all that work in preparation seminars, meeting with people at federal, state, and local levels, and briefing everyone involved, we expected then that we would get fair and impartial treatment in the media, which in fact we did. Of course, one paper decided to run a front-page article under a large title "Plants Given Killer Genes!" And this in spite of the fact, pointed out repeatedly by us, that these are nontoxic to humans, fish, mammals, birds, and other wildlife. But despite the occasionally sensationalistic story, we received very fair treatment from the media.

We then went ahead with our field trials and I'm happy to say, without incident. We feel that the public information campaign served its purpose despite the occasional article about killer genes. We had no concern from the public and in fact the only responses we got were very positive. There was a great deal of appreciation that the company had taken the time to have gone out and briefed the people on our activities.

The field trials themselves were very successful. This slide shows one of the control plants in our test plot. If you look closely at the top you can see what remains of the plant—petioles and stems. We happened to go out in a year when the tobacco hornworm pressure was about three times higher than at any time in the previous ten years.

Our *B.t.* plants, on the other hand, had some very small holes and we were able again to find dead caterpillars stuck on the leaves next to the holes. But damage to the plants was minimal. In fact, had we applied standard commercial criteria for spraying, these plants would have gone the entire season without requiring a single insecticide spray. Commercial tobacco growers in the county during that same season had applied five sprays to keep the hornworms down under control.

That's pretty much it for the formal part of my talk. I wanted to spend some time yet, as requested by the symposium's sponsors, to speculate on ways in which nonprofit national and international ag research centers might collaborate with the private sector in order to access this and related new technologies.

With situations like Dr. Beachy's, the charter of the university includes activities such as cooperating on a global scale in areas where there are no market returns. But you must understand that private companies are as constrained to work within their charters as you are to work within your institutional charters.

If we were to take stockholders' money, entrusted to us with the expectation of a return, and invest that in nonprofit ventures, the same thing would happen to our management as would happen to yours if you decided to work on rice and you were at an institute chartered to work on papaya. They would be replaced. So I spent a fair amount of time last night asking myself, "How might these two very different types of organizations work together and both benefit?" I do have some thoughts I'd like to throw out to you.

First, it's pretty clear that there are pieces of technology that are ready to be applied now to agronomic crops, such as the *B.t.* technology. Some are in the hands of the universities, a little more accessible; some at the private sector, a little less accessible. Secondly, the private sector is developing these with an eye towards the industrial markets.

An argument could be made that this kind of technology has even greater applicability and greater potential in the developing-nation markets. And the argument would go something like this: in the developing world the growers have less capability to purchase agrochemicals than in the industrialized countries, so they have fewer options. This technology does not require an investment by the grower in spray equipment and tractors, or the availability of aircraft to spray on insecticides. This technology would not require any training on the part of the growers in handling and properly disposing of potentially toxic chemicals. And finally, from a more societal viewpoint, a country with an agricultural base that had an option between controlling insects with this kind of technology versus chemical technology finds itself not being required to invest large amounts of capital in pesticide production and distribution facilities.

So there are a number of arguments that say this kind of technology actually is more suited by its nature to use in developing nations than in industrial nations, and yet the fact that patent protection exists and markets are more lucrative in the industrial West means that the companies with this technology are constrained to focus it towards those markets. That's kind of a sad paradox. And I've got a few thoughts I'd like to share with you—ways that we might interact to get this technology into the developing nations.

The key, I think, is the fact that there is additional research needed, additional resources needed, to take the genes that have been demonstrated in tobacco and tomato and test crops, and get them into cassava, papaya, and other crops. There is regeneration technology and transformation technology. There is a fair amount of

research and breeding, and then production and distribution that need to be done. And the second key is there needs to be some sort of incentive to the companies to collaborate. And how do we get to those two?

Well, the research side is rather straightforward. There are, and many of you are representatives of, national and international research and breeding operations that have exactly that capability—to take this technology from a Rohm and Haas, a University of Washington, and apply it to cassava, papaya, and other Third World crops. So you have something to offer when you come to the bargaining table. Where it gets more difficult is finding an incentive to the company to collaborate. That's a little tougher, but I do have a thought or two on that as well.

Developing nations need to promote domestic industries as well as meet the needs of their growers. Seed industries, by comparison to other types of industries, are fairly easy to get into. It does not take much more than research and distribution, frankly, to build a national seed company. Unlike a petrochemical complex or a computer industry, you do not need to have a technically based infrastructure. You do not need large support industries. You do not require heavy up-front capital investment. Your basic needs are an agricultural base and breeding research.

If one were to approach a company like Rohm and Haas and say, "Listen, we have the research capability to take your technology, put it into papaya, guava, and seven other crops that are important to us, and the country is willing to set up a national seed company to distribute this at low cost to our growers. And we will provide to your company as compensation for your contribution, a minority share interest in that national seed company," I think they might find this kind of approach attractive. At least they would certainly sit down and think about it. After all, these are markets that these companies normally would not go into anyway, so they're looking at no loss and perhaps a small gain.

It provides the country with the ability to offer their growers needed products at low cost while also building a base for a national seed company, a new domestic industry. It offers companies the chance to realize some return from markets that typically are closed to them either by virtue of the lack of patent protection or by the small size and lack of distribution systems. This would be especially true for those crops, like cassava, for which industrial seed companies do not breed anyway. And most companies enjoy seeing their products used to help people, a motivation you should not underestimate. In a properly structured collaboration, everybody should benefit.

An alternative approach might be a simple, old-fashioned horse trade. Although a breeding center might not have the fancy technology to genetically engineer new genes into crops, you certainly conduct a great deal of basic research and genetics. These are the sources of new genes of economic importance. You might, for example, after identifying a gene in cassava leaves that produces an antifungal agent, approach a company with this kind of deal: "We'll provide the information and genetic material, you do the genetic engineering we want, and you can have rights to the results for the industrial world's crops and we'll keep rights to the results for our crops and our regions."

Now, if I were to outline either proposal on a board, we could shoot it full of holes in a few minutes. I'm offering these thoughts, by no means as proposals, but as examples of a very important message I'd like to leave you with.

The private sector's mind-set is an extraordinarily flexible one. If they have something of value to someone else and they can get something back for it, they will work with you to brainstorm through ten other schemes until you find one that gives both parties what they need.

So be innovative when you think about these things. Rule nothing out, because the people in the industrial sector will not. They will look at every possible option to work together. If you come to a company and say, "You have something we need and there is something here that you might benefit from," they will work hard to find a way to proceed. That is perhaps the greatest strength of the private sector.

I hope those thoughts will stimulate discussion of other ideas on collaboration with industry, and I look forward to hearing your thoughts as this conference continues.

Application of Restriction Fragment Length Polymorphism to Plant Breeding

D.P. West, J.H. Cramer, J. Romero-Severson,
Y. Ma, M. Murray
Agrigenetics Corporation
Madison, Wisconsin

Breeders generate genetic variation by crossing unlike types, and then attempt to isolate individuals with particularly desirable combinations of the parental genes. The new assortment of genes is produced on the DNA level by the exchange of chromosomal segments (recombination) which occurs during meiosis. Because of multiple recombination, progenies are genetic mosaics of their parents' chromosomal segments (Figure 1). In the past, only a few recombination events were detectable at any one point in time and only in special genetic stocks.

Recombination has, therefore, been a black box to breeders who must deal at the phenotypic level of variation. If it were possible to tag or mark a segment of a chromosome, one could effectively follow genes located on that segment through the recombinational process. The idea of using genetic markers as tags for genes of interest is not new (see Edwards *et al.*, 1987). Economic application of genetic markers has been limited historically because no one system of markers provided all of the following necessary characteristics:

1) Selectively neutral, nondeleterious
2) Ubiquitous, allowing saturated coverage of the genome
3) High level of polymorphism
4) Easily detectable variation
5) Codominant, unaffected by environment

Markers based on DNA restriction fragment length polymorphism (RFLP) satisfy these criteria and are being used extensively to build detailed genetic maps in a wide variety of crop species (e.g., Burr *et al.*, 1988; Helentjaris *et al.*, 1986; Bernatzky and Tanksley, 1985; Landry *et al.*, 1987; Murray *et al.*, 1988; see Figure 2).

What Is Restriction Fragment Length Polymorphism?

Restriction endonucleases cleave DNA at specific nucleotide sequences distributed throughout the genome. A corn nucleus contains about 6 billion base pairs of DNA, and hundreds of thousands of different fragments ranging in size from several hundred to 20,000 base pairs are produced when the DNA is cut with an

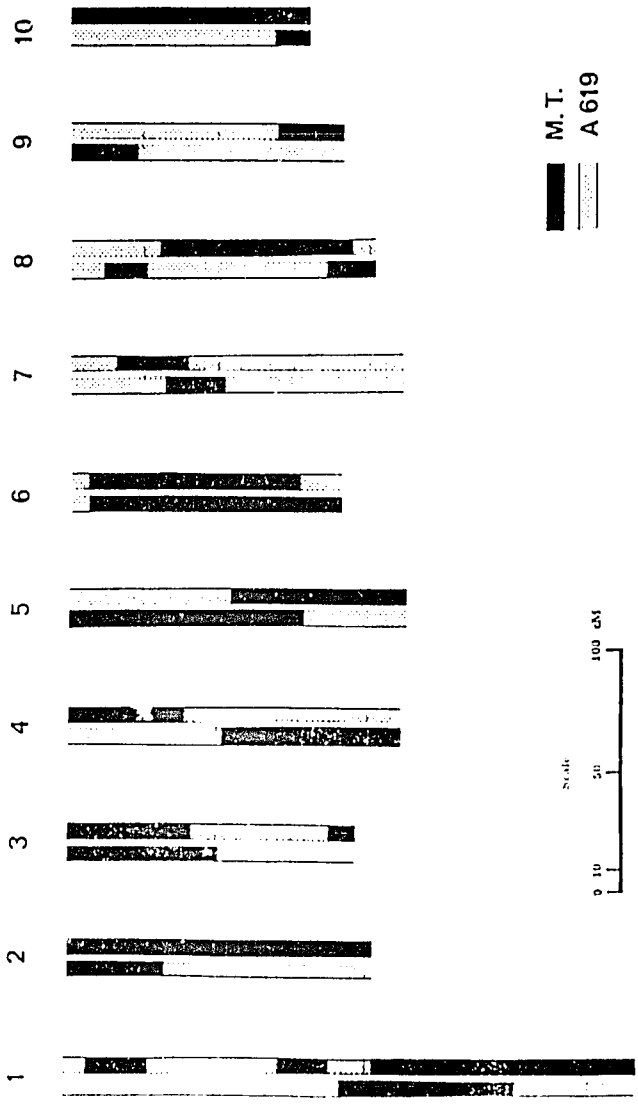


Figure 1. Riflotype of an Individual Maize Plant.

This figure depicts the riflotype of one of the Mangelsdorf's Tester X A619 F2 individuals used to construct the Maize RFLP map shown in Figure 2. Segments derived from the Mangelsdorf's Tester parent are shown with solid bars while those from the A619 parent are shown with stippled bars. This figure was constructed so as to minimize double recombinant chromatids and represents only one of several possible solutions.

enzyme such as *Eco*RI. The process of Southern hybridization makes it possible to identify any one fragment of interest among the myriad of other fragments (Figure 3). The restriction fragments can be resolved according to size using gel electrophoresis. They are then transferred out of the electrophoresis medium onto a membrane support such as nitrocellulose or nylon. Small, isotopically-labeled, nucleic acid fragments are used to "probe" the membrane because under appropriate conditions, they will hybridize specifically to homologous DNA sequences immobilized on the membrane, thus revealing a specific restriction fragment's length or size.

Probes are made by cloning random pieces of DNA from the organism of choice and then selecting those suitable for use as RFLP markers. Although probes that hybridize to repetitive sequences can be useful, those hybridizing to single copy segments are preferred for genetic mapping because of ease of interpretation. Most poly(A) RNA is transcribed from single copy sequences. Therefore, cDNA libraries prepared from poly(A) RNA are an excellent source of single copy clones. A more recent innovation is to clone those sequences that are cleaved by the methylation-sensitive restriction enzyme PstI and exploit the observation that undermethylated DNA is highly enriched for single copy sequences in plants (Burr *et al.*, 1988). Probes are typically labeled with radioisotopes but nonradioactive detection systems are under intensive development.

A given DNA probe is generally useful within a species and may often also be useful among closely related species (Tanksley *et al.*, 1988). For a probe to be useful, it must hybridize to fragments of different lengths in the parents of interest. Although restriction fragments produced with one restriction endonuclease may not be polymorphic, the possibility of finding useful polymorphisms with a particular probe increases as DNA digested with a spectrum of other enzymes is examined. The frequency of easily detectable restriction fragment length polymorphisms is affected by factors such as the size and evolutionary history of the particular plant genome. In organisms with high levels of genetic diversity, such as maize, it is possible to construct genetic maps using few enzymes. Where genetic diversity is limited, extensive surveys are necessary to find enzyme/probe combinations that reveal polymorphism.

Restriction fragment size is a stable genetic attribute of an individual or genotype (Evola *et al.*, 1986). A size difference, or polymorphisms, between two parents will segregate in their progeny like alleles of a gene. Classical segregation analysis (Mather, 1938) can be used to construct a genetic map that locates the RFLP

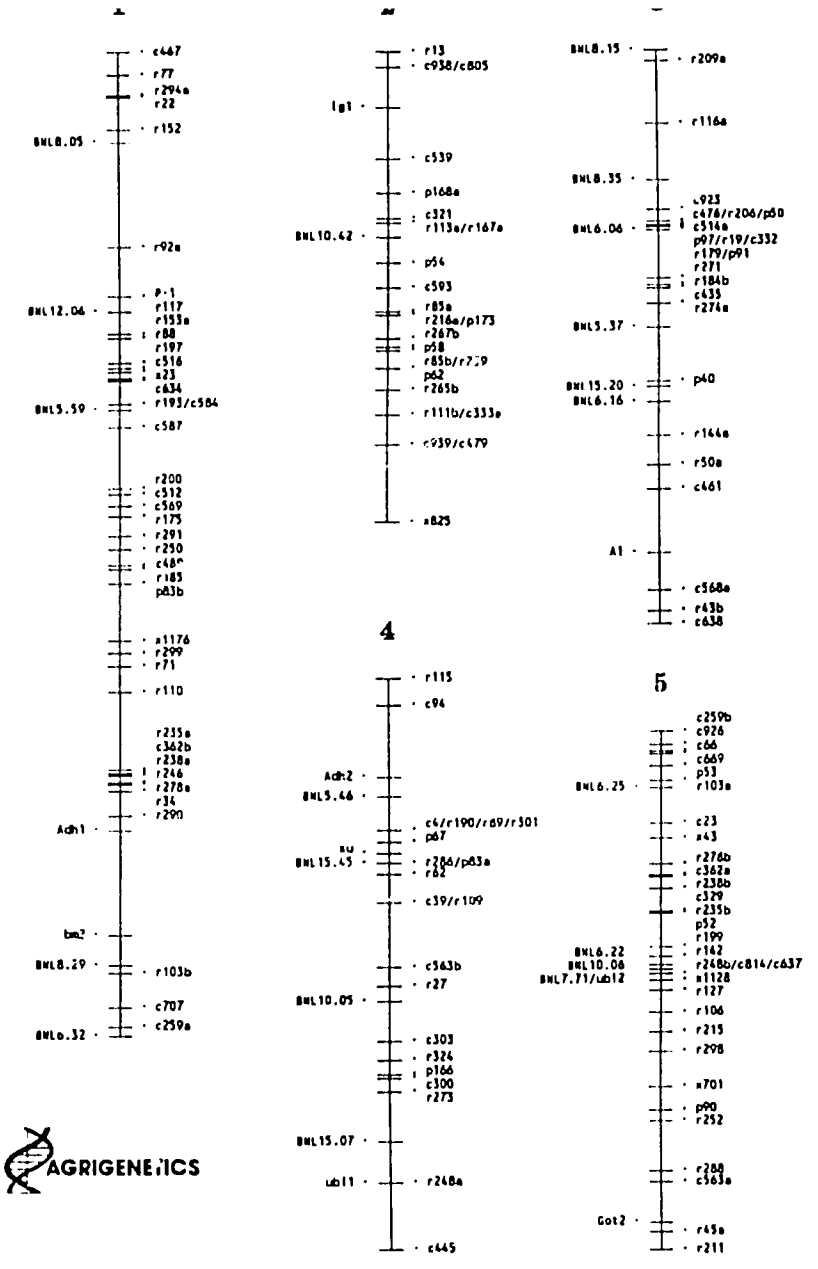
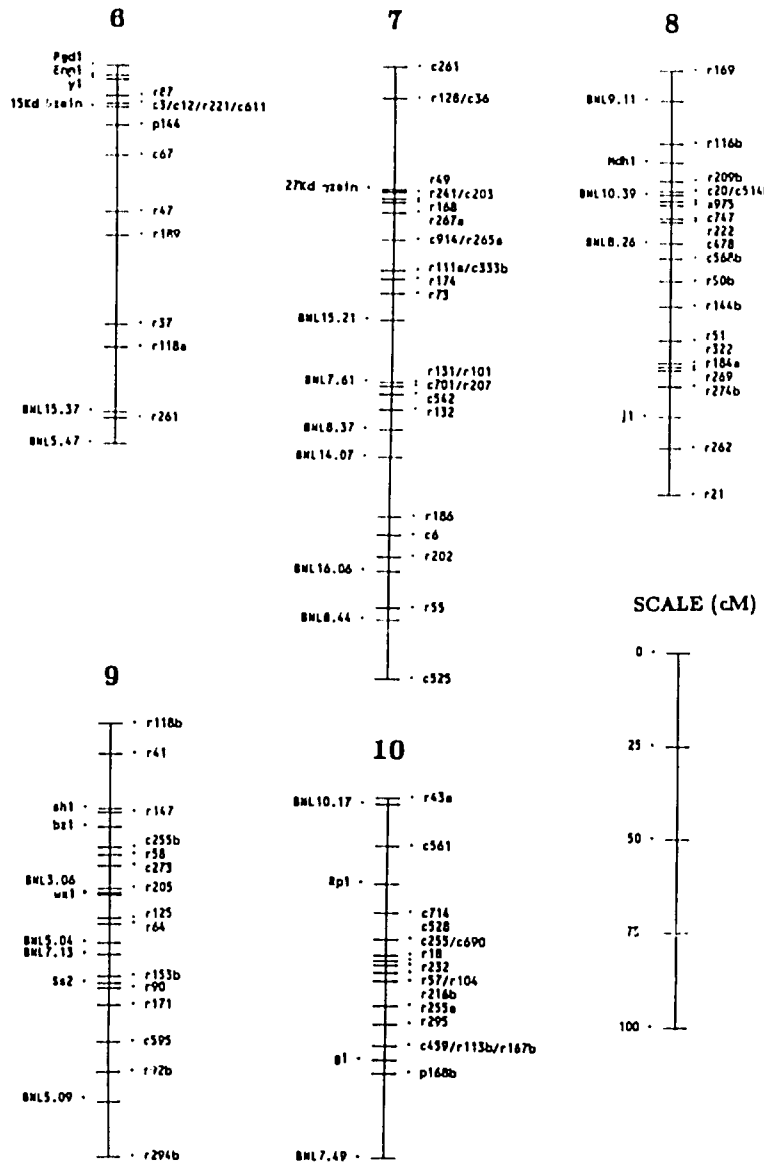


Figure 2. An RFLP Linkage Map for Maize.

This map was constructed using 90 F2 individuals derived from a cross between Mangelsdorf's Tester and A619. Established genetic markers or cloned DNA sequences obtained from other researchers are shown on the left side of each chromosome. The prefix BNL denotes RFLP markers obtained from Dr. Ben Burr at Brookhaven



National Laboratory. RFLP markers developed at Agrigenetics are shown on the right. Prefixes to these markers designate the type of clone used: c, dark-grown coleoptile total poly(A) cDNA clones; r, root polysomal poly(A) cDNA clones; p, PstI genomic DNA fragments; x, XhoI genomic DNA fragments. Independent markers between which no recombinants were observed are shown on the same line.

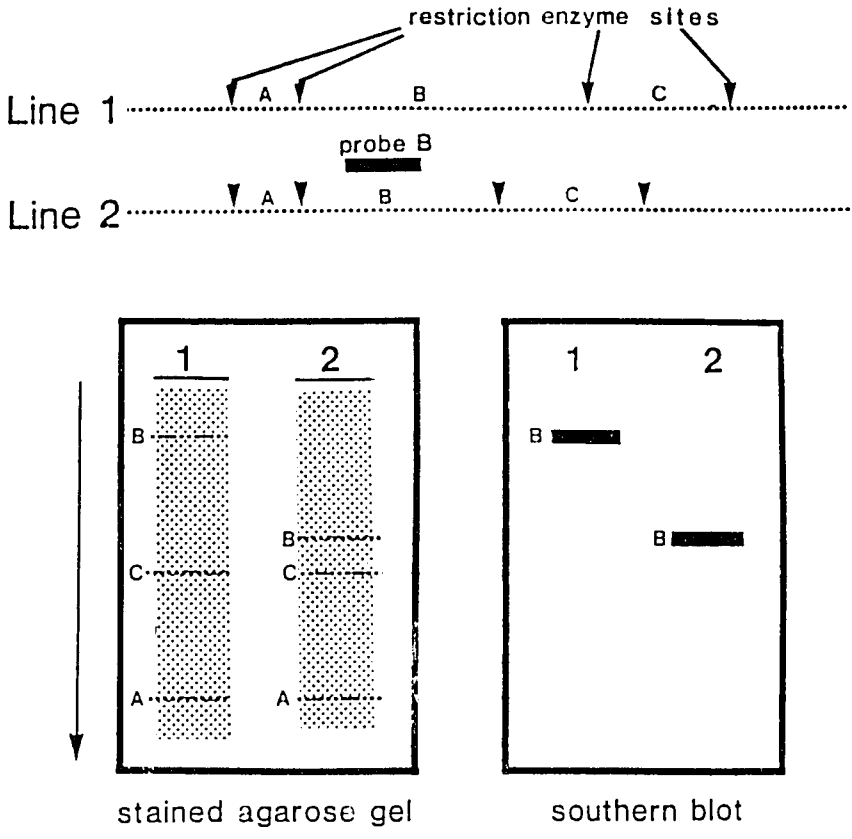


Figure 3. Fundamentals of RFLP analysis.

The DNA fragments produced after restriction endonuclease digestion of genomic DNA from two hypothetical lines are separated according to size by agarose gel electrophoresis. Because any one fragment may represent less than one part in several hundred thousand, it is not visible when the total DNA is visualized by staining. To detect a specific fragment, the DNA is transferred out of the gel onto a membrane and hybridized to an isotopically-labeled probe, probe B in the example. Autoradiography is used to determine the position of genomic DNA fragments homologous to the probe. Probe B reveals polymorphism between the two lines whereas probes for A or C would not.

markers with respect to each other. The analysis is performed on progeny constructed for that purpose from a cross of unrelated parents. The mathematics of the linkage analysis are adjusted appropriately for an F2, backcross, or recombinant inbred progeny. In species for which classical maps already exist, the use of parents such as Mangelsdorf's tester (maize; Murray *et al.*, 1988) and Rick's isozyme stocks (tomato; Bernatzky and Tanksley, 1985) in the production of a mapping progeny has facilitated cross-referencing the classical with the emerging RFLP map. Aneuploid stocks are also useful in assigning probes to chromosome (Helentjaris *et al.*, 1986).

Practical Uses of RFLP Analysis

Restriction fragment length polymorphism is a powerful tool that has many practical applications in plant breeding (Table 1). RFLP can be used in essentially all the areas where isozymes have been suggested previously (Stuber *et al.*, 1982; Smith, 1984), but with significantly higher resolution.

It is possible to begin using RFLP long before one has a saturated linkage map. For example, a composite pattern or fingerprint which differs among genotypes can often be created with relatively few probes. Probes that hybridize to moderately repetitive sequences can also be useful for producing such genotype-specific patterns.

Table 1. Applications of RFLP Technology.

DNA Fingerprinting:
Identity (plant variety protection, litigation)
Relatedness (pedigree analysis, combining ability)
Recurrent parent recovery
Genetic purity testing
Trait mapping:
Quantitative and/or polygenic traits
Exploiting unadapted germplasm
Engineered genes
Marker-based breeding:
Rapid line conversion (introgression management)
Gene stacking (disease resistance, quality factors)
Early tester selection

However, if the probes used in a fingerprint are mapped, then the extent and location of differences between genotypes is known. DNA fingerprints will inevitably be important in establishing line identity, plant variety protection, and patent protection. For hybrid breeding programs, choice of tester may ultimately be guided by the ability to trace lines of descent and determine relatedness with RFLP.

RFLP technology is being used to map chromosomal regions involved in traits of economic value. Trait mapping requires construction of a segregating progeny with sufficient phenotypic contrast for the trait of interest. Phenotypic data are obtained using conventional assays and experimental designs. The genotypic constitution of individual progenies is determined using RFLP markers. Phenotypic data is then regressed on genotypic data to identify chromosomal regions that contribute significantly to the variance in phenotype. The breeder can then select desired genotypes using the identified RFLP markers.

Generally, to improve the fitness of their crops, breeders must manipulate traits determined by the interactions of many genes. RFLP can facilitate this manipulation by permitting visualization of individuals of a population as mosaics of parental chromosome segments. If, for example, the objective is to transfer a major disease resistance gene from an unadapted donor stock to an elite line, the breeder will want to recover all of the performance of the elite recurrent parent. Our analysis of certain backcross conversions in maize revealed sizable unlinked genomic segments from the nonrecurrent parent persisting in the derived line. Linkage drag of markers within 30 map units around the desired gene was also noted (Ma *et al.*, 1988). Breeders can use RFLP in conjunction with backcrossing to identify individuals that have the optimum recurrent parent recovery and include the desired donor parent locus with minimum linkage drag.

When the objective is to gather several specific alleles into one genotype, RFLP tags can be used to determine which individuals in a progeny carry all or most of the desired alleles. This will be useful for the "stacking" of multiple resistance genes. As the number of genes increases, the population size theoretically required to obtain one individual with all the desired genes grows exponentially. RFLP analysis permits the detection of those genotypes that, through controlled mating, could produce the desired combination.

Present RFLP technology is not yet capable of efficiently handling the large numbers of individuals commonly dealt with by plant

breeders. Technical improvements in probe detection systems, mechanization, data acquisition, and computer software will be essential before the full impact of RFLP on plant breeding can be achieved. As RFLP technology becomes a routine economic germplasm management tool, the reservoir of genetic material available in land races, random germplasm accessions, and wild relatives of our crops will become more accessible and manageable. However, for these populations to be utilized, they must be genetically structured and phenotypically characterized with the standard approaches of conventional plant breeding. The preparation and characterization of germplasm resources is time- and labor-consuming and should be initiated in advance in anticipation of eventual RFLP analysis.

Literature Cited

Burr, B.A., F.A. Burr, K.H. Thompson, M.C. Albertsen, and C.W. Stuber. 1988. Gene mapping with recombinant inbreds in maize. Genetics 118:519-526.

Bernatzky, R. and S.D. Tanksley. 1985. Toward a saturated linkage map in tomato based on isozymes and random cDNA sequences. Genetics 112:887-898.

Edwards, M.D., C.W. Stuber, and J.F. Wendel. 1987. Molecular-marker-facilitated investigations of quantitative trait loci in maize. 1. Numbers, genomic distribution, and types of gene action. Genetics 116:113-125.

Evola, S.V., F.A. Burr, and B. Burr. 1986. The suitability of restriction fragment length polymorphisms as genetic markers in maize. Theor. Appl. Genet. 71:765-771.

Helentjaris, T., M. Slocum, S. Wright, A. Schaefer, and J. Nienhuis. 1986. Construction of genetic linkage maps in maize and tomato using restriction fragment length polymorphisms. Theor. Appl. Genet. 72:761-769.

Helentjaris, T., D.F. Weber, and S. Wright. 1986. Use of monosomics to map cloned DNA fragments in maize. Proc. Natl. Acad. Sci. 83:6035-6039.

Landry, B.S., R.V. Kesseli, B. Farrara, and R.W. Michelmore. 1987. A genetic map of lettuce (*Latuca sativa* L.) with restriction fragment length polymorphism, isozyme, disease resistance, and morphological markers. Genetics 116:331-337.

Ma, Y., D.P. West, J.H. Cramer, J. Romero-Severson, and M. Murray. 1988. Near-isogenic line revisited (in preparation).

Mather, K. 1938. The measurement of linkage in heredity. Metheun & Co. Ltd., London.

Murray, M., J. Cramer, Y. Ma, D. West, J. Romero-Severson, J. Pitas, S. DeMars, L. Vilbrandt, J. Kirschman, B. McLeester, J. Schilz, and J. Lotzer. 1988. Agrigenetics maize RFLP linkage map. Maize Genetics Cooperation Newsletter 62:89-91.

Smith, J.S.C. 1984. Genetic variability within U.S. hybrid maize: multivariate analysis of isozyme data. Crop Sci. 24:1041-1046.

Stuber, C.W., M.M. Goodman, and R.H. Moll. 1982. Improvement of yield and ear number resulting from selection at allozyme loci in a maize population. Crop Sci. 22:737-740.

Tanksley, S.D., R. Bernatzky, N.L. Lapitan, and J.P. Prince. 1988. Conservation of gene repertoire but not gene order in pepper and tomato. Proc. Natl. Acad. Sci. (in press).

Development and Field Testing of Crops Improved Through Genetic Engineering

Xavier Delannay, Robert T. Fraley, Stephen G. Rogers, Robert B. Horsch, Ganesh M. Kishore, Roger N. Beachy, Nilgun N. Tumer, David A. Fischhoff, Harry J. Klee and Dilip M. Shah
Monsanto Company
St. Louis, Mo.

Introduction

A lot of progress has been made since the first transgenic tobacco plants were produced in 1983. A growing number of crop species have proved amenable to transformation, mostly through the *Agrobacterium* transformation system, and a number of potentially useful genes have been identified and introduced into plants. Although most of the work with transgenic plants has taken place so far only in greenhouse and laboratory environments, approval was recently granted to various research groups for testing the first transgenic plants on a larger scale in the field.

Testing transgenic plants in the field permits a more thorough evaluation of the potentials of genetic engineering for crop improvement, both in terms of the agronomic use of the engineered genes, and in terms of the agronomic adaptation of the transgenic plants in a normal field environment. The field tests are currently performed under close scrutiny from the regulatory agencies, and are subject to various restrictions aimed at preventing a premature escape of the engineered gene in the environment. However, the information gained from those tests will progressively alleviate the environmental concerns expressed by some groups, so that the restrictions can be removed one by one, and the benefits of biotechnology can finally be fully applied to agriculture.

In this presentation, a brief overview of the *Agrobacterium* transformation techniques will be given, followed by an update on the recent progress in gene development at Monsanto, and a description of the recent field tests conducted near Jerseyville, Illinois. Finally, we will discuss the way Monsanto plans to commercialize plant biotechnology products, so that their benefits can be made widely available to the farmers.

Plant Transformation Technology

Since their first successful application in 1983, the *Agrobacterium tumefaciens* transformation systems have been widely used for

transformation of dicotyledonous species, and have been adapted to a growing number of crops. More than twenty species of plants have been transformed so far, and the list will probably continue to grow within the next few years until all major dicotyledonous species can be manipulated using that technology. Progress in that area has depended largely on three major factors:

1) *Development of vectors based on the Agrobacterium tumefaciens Ti plasmid system*

   Agrobacterium tumefaciens has a natural capability to insert a part of its DNA, known as T-DNA, into plants cells (Bevan *et al.*, 1982; Schell and van Montagu, 1983; Fraley *et al.*, 1986). Natural T-DNA contains two main classes of genes; phytohormone genes responsible for the tumor formation characteristic of the crown gall disease, and opine genes that lead to the synthesis of specific compounds, nopaline or octopine, used by the bacterium for its growth inside the plant. In the *Agrobacterium tumefaciens* transformation systems, the phytohormone genes have been removed, so that no tumor develops and normal plants can be regenerated from the infected explant through tissue culture. Those tumor genes have been replaced by synthetic genes that code for selectable or scoreable markers that will facilitate selection and identification of the transformed plant, and for gene products responsible for the new agronomic traits.

2) *Availability of multiple selectable markers*

   Neomycin phosphotransferase (NPT), which confer resistance to the antibiotics kanamycin and geniticin, have been used very extensively in plant transformation experiments, and are still among the most useful selectable marker genes (Herrera-Estrella *et al.*, 1983; Fraley *et al.*, 1983; Bevan *et al.*, 1983). More recently, many additional selectable markers were developed which complement NPT and extend the range of plants in which selection of transformants can be readily achieved. This development is especially important in crop species where NPT does not work very efficiently. Examples of such markers are hygromycin phosphotransferase (Lloyd *et al.*, 1986; Van den Elzen *et al.*, 1985) and gentamycin acetyltransferase (Hayford *et al.*, 1988).

3) *Improvements in plant regeneration systems*

   The first transformed plants were obtained using protoplast systems, but the use of those techniques presents substantial

technical difficulties and is limited to a few species for which protoplast regeneration is possible. The large increase in number of successful crop transformations over the past few years is largely due to the use of tissue explants such as leaves (Horsch *et al.*, 1985), stems (Lloyd *et al.*, 1986) or cotyledons (McCormick *et al.*, 1986) for transformation with *Agrobacterium* and subsequent plant regeneration. Table 1 lists the transgenic plants that have been reported to be transformable as of early 1988.

Table 1. Species in Which Transgenic Plants Have Been Reported.

alfalfa	flax	poplar
Arabidopsis	lettuce	potato
asparagus	lotus	sugarbeet
cabbage	oil seed rape	sunflower
carrot	pear	tobacco
celery	peas	tomato
cotton	petunia	white clover
cucumber		

With the targeting of *Agrobacterium* strains, vectors and selectable markers to particular regeneration systems, it seems that all dicotyledonous crop species should eventually be amenable to transformation within the next few years.

Identification of Genes Useful For Crop Improvement

While transformation systems were being developed, a large amount of research was carried out over the past few years to identify and isolate genes that could be useful for crop improvement. The most exciting opportunity offered by biotechnology is to provide the plant breeders with new sources of genes that are not available through classical means. Those genes can come from a wide diversity of living organisms (other plant species, bacteria, viruses, animals), and can be subjected to specific modifications that enhance or modify their characteristics. The following discussion on gene development will concentrate on Monsanto research programs.

Virus tolerance

Disease resistance has been an important focus of research by plant pathologists and breeders for a very long time, and a large number of sources of tolerance to specific races of various pathogens have been developed over the years. However, there are still a number of pathogens for which no source of tolerance is known, and most

available tolerances are specific to particular races and subject to break down as new diversity appears in the pathogen population. Biotechnology can offer new avenues for disease resistance that may lead to a broader range of protection that could be less sensitive to changes in the genetic makeup of the pathogen populations. Encouraging examples are the new avenues for virus resistance that were recently developed through bioengineering.

McKinney (1929) observed that tobacco plants infected with a strain of a virus often resist infection by a second related strain. Although the mechanism of this phenomenon, called cross-protection, was not fully understood, several investigators suggested that protection from viral diseases could be achieved through gene transfer methods (Hamilton, 1980; Sequira, 1984; Palutaikis and Zaitlin, 1984; Beachy *et al.*, 1985; Bevan *et al.*, 1985; Sanford and Johnston, 1985). Powell Abel *et al.* (1986) developed a vector containing a cDNA encoding the coat protein gene of the U1 strain of tobacco mosaic virus (TMV), flanked by the 35S promoter from cauliflower mosaic virus (CaMV) and a polyadenylation signal from the *Agrobacterium tumefaciens* nopaline synthase gene. After introduction into tobacco and tomato plants, this synthetic gene induced the production of high levels of TMV coat protein in the cells (0.05 to 0.1% of total soluble cell protein). When the transgenic plants were infected with two different strains of TMV, symptom development was either totally inhibited, or much slower than that observed in the nontransformed controls (Nelson *et al.*, 1988).

A similar protection was obtained when the phenomenon of genetically engineered cross-protection was extended in our laboratory to other viruses such as alfalfa mosaic virus (AlMV) (Tumer *et al.*, 1987), potato virus X (PVX) and cucumber mosaic virus (CMV). In all those cases, cloning of the coat protein gene and introduction of that gene into plants resulted in a significant level of protection against the corresponding virus.

Insect tolerance

Another area where genetic engineering can have important applications in crop improvement is the development of insect tolerant plants. Most of the potential at this time seems to reside in the utilization of genes coding for the insect toxin produced by the spores of *Bacillus thuringiensis* (B.t.). Although most B.t. strains are toxic to lepidopteran larvae (Dulmage, 1981), a few have been found to have activity against dipteran (Goldberg and Margalit, 1977) or coleopteran (Krieg *et al.*, 1983; Herrnstadt *et al.*, 1986) larvae.

Fischhoff *et al.* (1987) constructed a chimeric gene consisting of a fragment (amino acids 1 through 725) of a lepidopteran-specific oxin from B.t. subsp. *kurstaki* HD-1 (Watrud *et al.*, 1985), flanked by the CaMV 35S promoter and the 3'-end of the nopaline synthase gene. When introduced into tomatoes, the plants expressed the B.t. toxin gene and were toxic to larvae of tobacco hornworm (*Manduca sexta*), which are known to be sensitive to the B.t. toxin. All the larvae applied to transgenic plants died within a few days, and the plants showed little feeding damage. In contrast, larvae applied to control plants developed normally and caused severe damage to the plants.

Herbicide tolerance

Herbicide tolerance is another area that has been actively pursued by bioengineers over the past few years. It will be of great importance to the farmers by providing more efficient, cheaper, and environmentally more-acceptable ways to control weeds in cultivated crops. Most of the activities have centered on a group of herbicides (Roundup® , sulfonylureas, imidazolinones, and others) whose particular mode of action permitted a rapid determination of key target genes.

Glyphosate, the active ingredient of Roundup® herbicide, is a broad-spectrum, nonselective herbicide used extensively in noncrop situations. It is rapidly absorbed by foliar tissue and translocated to various plant organs. It is also rapidly metabolized in the soil and has no residual activity. Glyphosate kills the plants by blocking a shikimate pathway enzyme, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), involved in aromatic amino acid biosynthesis (Steinrucken and Amrhein, 1980; Mousdale and Coggins, 1984; Rubin *et al.*, 1984; Nafziger *et al.*, 1984).

Glyphosate-tolerant plants were developed at Monsanto through the high-level expression of a plant EPSPS gene isolated from a glyphosate-resistant suspension cell line of *Petunia hybrida* (Shan *et al.*, 1986). The gene coded for a precursor polypeptide that had an amino-terminal sequence of 72 amino acids associated with the actual EPSPS enzyme. The amino-terminal sequence, called "transit peptide," is responsible for the movement of the enzyme into the chloroplast, where EPSPS activity actually takes place (della-Cioppa *et al.*, 1986). When this gene, under control of the CaMV 35S promoter, was introduced into petunia cells, the resulting callus grew normally on concentrations of glyphosate that would normally totally inhibit proliferation of nontransformed cells. Plants regenerated from the transformed calluses survived applications of 0.9 kg/ha of Roundup® , approximately four times the quantity that

would kill the control plants, but had a reduced growth compared to unsprayed controls.

In further experiments, transformation was carried out with a mutant EPSPS gene that produces an enzyme that is 6000-fold less sensitive to glyphosate than the wild-type enzyme. Resulting tobacco plants had a significantly improved level of tolerance to glyphosate: they did not show any obvious signs of injury and produced normal flowers and seed when sprayed with 0.9 kg/ha of glyphosate.

Field Evaluation of Transgenic Plants

As more and more success was obtained with plant transformation and the new genes showed promising levels of activity under laboratory and greenhouse conditions, it became essential to test the new plants in the field to better determine the potentials of using biotechnology for crop improvement. The two main questions to be answered in such experiments were whether the level of gene activity observed in the greenhouse would be maintained and sufficient under variable field conditions, and whether the transformation process could lead to changes in the agronomic characteristics of the plants (yield, general growth, quality), besides the introduction of the improved gene.

In 1987, Monsanto Company obtained permission from the U.S. Department of Agriculture to field test genetically engineered tomato plants carrying the glyphosate tolerance, insect tolerance and virus tolerance genes described above. The site chosen was a farm located 5 km outside the farming community of Jerseyville, Illinois. Although the conditions of the site were favorable to tomato growth, it was not located in a commercial tomato production area. The local community and state officials were briefed in advance, and were very supportive of the experiment. The main restrictions for this first field release of transgenic tomatoes were aimed at preventing escape of any part of the plants (pollen, vegetative parts, or fruit and seed) from the experimental area: 1) the experiment was more than 400 meters away from the closest possible garden-grown tomatoes, 2) the field was fenced to protect the plants from animals that could disperse some of the material, 3) the fruits were harvested at breaker stage, before they become attractive to birds and other animals that could disperse the seed after feeding. Over 9,000 tomato plants (genetically engineered and controls) were planted during the month of June; agronomic practices closely followed commercial procedures for processing tomatoes.

In the tobacco mosaic virus experiment, transgenic lines and untransformed controls were submitted to three treatments: infection with TMV strain U1 at two different times of growth, and untreated controls. All infected control plants showed virus symptoms within a few weeks after treatment, while less than 5% of the transgenic tomatoes containing the coat protein gene displayed symptoms. When symptoms did appear, they were mild and restricted to the inoculated leaves. Biochemical tests confirmed the lack of systemic spread of the virus in the transgenic plants. Untreated transgenic plants yielded similarly to the untreated controls, showing that the transformation process did not affect the normal agronomic characteristics of the plants. However, a significant yield reduction (23-33%) was detected in the control plants after virus inoculation, whereas inoculated transgenic plants did not differ in yield from the uninoculated controls. The extent of the yield loss on inoculated nontransgenic plants was somewhat surprising because U1 caused only very mild visual symptoms on the plants. This may indicate that "subclinical" levels of virus disease in plants may cause significant but usually undetected yield reductions.

The insect tolerance experiment subjected plants to three treatments: infestation with eggs of tobacco hornworm (*Manduca sexta*), infestation with eggs of tomato fruitworm (*Heliothis* zea), or non-infested controls. Nontransgenic plants were severely to completely defoliated a few weeks after infestation with *Manduca sexta*, whereas the only damage on the transgenic plants expressing the B.t. gene was limited to a few small holes near the infestation site representing the initial feeding by the newly hatched larvae before they became intoxicated by the toxin present in the leaves. *Heliothis* zea feeds primarily inside the fruits, so that the results were mostly visible at harvest time. Fruit damage on nontransgenic control plants was 17-23% (depending on whether infestation was natural or inoculated), while only 4-9% of the fruit on transgenic plants showed fruitworm damage. These field results with the tomato fruitworm were better than what had been expected based on greenhouse results, suggesting that the B.t. insecticidal protein gene may be more effective under field conditions.

In the case of glyphosate-tolerant plants, 22 independent transgenic lines were sprayed with various rates of Roundup® , along with the controls. Most unsprayed transgenic plants yielded similarly to the nontransgenic controls, showing that transformation did not affect the growth of the plants. Transgenic plants survived applications of more than 1 kg/ha a.e. of Roundup® , while control plants were rapidly killed at that rate. The treated plants displayed good vegetative tolerance, but showed reduced yields relative to unsprayed controls. Given the extreme sensitivity of tomatoes to

glyphosate, these results are very encouraging, and commercial levels of tolerance to Roundup® should be achievable once improvements on gene construction have been performed.

Commercialization of Biotechnology Products

As biotechnology is getting close to developing agronomically acceptable products, several ways are being devised to deliver those products to the marketplace. As for conventional breeding, the new genetic material will be available to the farmer in the form of seed. This means that the new technology will be directly accessible to the end user, without required knowledge of any new techniques. The mass production and distribution of transgenic material will, therefore, be best handled by the seed companies, using their long-time experience in this area. Biotechnology companies, thus, have several options: develop a seed business of their own, take control of existing seed companies, or enter into licensing agreements with independent seed companies for introduction and commercialization of the new genes in their germplasm. Monsanto has determined that the optimum way to access a large portion of a crop's germplasm, and make the new traits available to a large majority of users in the minimum amount of time, is through the licensing option. In this situation, the seed company remains proprietor of its germplasm, while the biotechnology company remains the owner of the gene. Depending on contractual agreements, a certain amount of royalty is paid by the seed company to the biotechnology company for the commercialization of the gene in the seed company's germplasm.

References

Beachy, R.N., Powell Abel, P., Oliver, M.J., De, B., Fraley, R.T., Rogers, S.G. and Horsch, R.B. 1985. Potential for applying genetic transformation to studies of viral pathogenesis and cross protection. In: "Biotechnology in Plant Science," M. Zaitlin, P. Day and A. Hollaender, Eds. Academic Press, Orlando, Florida, pp. 265-275.

Bevan, M., Van, M. and Chilton, M.D. 1982. T-DNA of the *Agrobacterium* Ti and Ri plasmids. *Annual Review of Genetics*, 16: 357-384.

Bevan, M., Favell, R.B. and Chilton, M.D. 1983. A chimeric antibiotic resistance gene as a selectable marker for plant cell transformation. *Nature*, 304: 1984-187.

Bevan, M.W., Mason, S.E. and Goelet, P. 1985. Expression of tobacco mosaic virus coat protein by a cauliflower mosaic virus promoter in plants transformed by *Agrobacterium*. *EMBO J.*, 4: 1921-1926.

della-Cioppa G., Bauer, S.C., Klein, B.K., Shah, D.M., Fraley, R.T. and Kishore, G.M. 1986. Translocation of the precursor of 5-enolpyruvylshikimate-3-phosphate synthase into chloroplasts of higher plants *in vivo*. *Proc. of the Nat. Acad. Sci., USA*, 83: 6873-6877.

Dulmage, H.T. 1981. Insecticidal activity of isolates of *Bacillus thuringiensis* and their potential for pest control. In: "Microbial Control of Pests and Plant Diseases 1970-1980," H.D. Burges, Ed., Academic Press, New York, pp. 193-222.

Fischhoff, D.A., Bowdish, K.S., Perlak, F.J., Marrone, P.G., McCormick, S.M., Niedermeyer, J.G., Dean, D.A., Kusano-Kretzmer, K., Mayer, E.J., Rochester, D.E., Rogers, S.G. and Fraley, R.T. 1987. Insect Tolerant Transgenic Tomato Plants. *Bio/Technology*, 5: 807-813.

Fraley, R.T., Rogers, S.G., Horsch, R.B., Sanders, P., Flick, J., Adams, S., Bittner, M., Brand, L., Fink, C., Fry, J., Galluppi, G., Goldberg, S., Hoffmann, N. and Woo, S. 1983. Expression of bacterial genes in plant cells. *Proceedings of the National Academy of Sciences U.S.A.*, 80: 4803-4807.

Fraley, R.T., Rogers, S.G. and Horsch, R.B. 1986. Genetic transformation in higher plants. *CRC Critical Reviews in Plant Sciences*, 4: 1-46.

Goldberg, L.J. and Margalit, J. 1977. A bacterial spore demonstrating rapid larvicidal activity against *Anopheles sergentii, Uranatenia unguiculata, Culex univittatus, Aedes aegypti* and *Culex pipiens*. *Mosquito News*, 37: 353-358.

Hamilton, R.I. 1980. Defenses triggered by previous invaders: Viruses. In: "Plant Disease," J.G. Horsfall and E.B. Cowling, Eds., Academic Press, volume 5, pp. 279-303.

Hayford, M., Medford, J., Hoffmann, N., Rogers, S. and Klee, H. 1988. *Plant Physiol.*, (in press).

Herrera-Estrella, L., DeBlock, M., Van Montagu, M. and Schell, J. 1983. Chimeric genes as dominant selectable markers in plant cells. *EMBO J.*, 2: 987-994.

Herrnstadt, C., Soares, G.G., Wilcox, E.R. and Edwards, D.L. 1986. A new strain of *Bacillus thuringiensis* with activity against Coleopteran insects. *Bio/Technology*, 4: 305-308.

Horsch R.B., Fry, J.B., Hoffmann, N.L., Eichholtz, D. Rogers, S.G. Horfmann, Fraley, R.T. 1985. A simple and general method for transferring genes into plants. *Science*, 227: 1229-1231.

Krieg, A., Huger, A.M., Langenbruch, G.A. and Schnetter, W. 1983. *Bacillus thuringiensis* var. *tenebrionis*, ein neuer, gegenuber Larven von Coleopteran wirksamer. *Pathotyp. Z Angew Entomol.*, 96: 500-508.

Lloyd, A.M., Barnason, A.R., Rogers, S.G., Byrne, M.C., Fraley, R.T. and Horsch, R.B. 1986. Transformation of *Arabidopsis thaliana* with *Agrobacterium tumefaciens*. *Science*, 234: 464-466.

McCormick, S., Niedermeyer, J., Fry, J., Barnason, A., Horsch, R. and Fraley, R. 1986. Leaf disc transformation of cultivated tomato (*L. esculentum*) using *Agrobacterium tumefaciens*. *Plant Cell Reports*, 5: 81-84.

McKinney, H.H. 1929. Mosaic diseases in the Canary Islands, West Africa and Gibraltar. *Journal of Agricultural Research*, 39: 557-578.

Mousdale, D.M. and Coggins, J.R. 1984. Purification and properties of 5-enolpyruvylshikimate-3-phosphate synthase from seedlings of *Pisum sativum*. *Planta*, 160: 78-83.

Nafziger, E.D., Widholm, J.M., Steinrucken, H.C. and Kilmer, J.L. 1984. Selection and characterization of a carrot cell line tolerant to glyphosate. *Plant Physiology*, 76: 571-574.

Nelson, R.S., Powell Abel, P. and Beachy, R. 1988. Fewer viral infection sites in transgenic tobacco plants expressing the coat protein gene of tobacco mosaic virus. *Virology*, (in press).

Palukaitis, P. and Zaitlin, M. 1984. A model to explain the "cross-protection" phenomenon shown by plant viruses and viroids. In: "Plant-Microbe Interactions. Molecular and Genetic Perspectives," T. Kosuge and E.N. Nester, Eds. Macmillan Press, New York, volume 1, pp. 420-429.

Powell Abel, P., Nelson, R.S., De, B. Hoffmann, N. Rogers, S.G., Fraley, R.T. and Beachy, R.N. 1986. Delay of disease develop-

ment in transgenic plants that express the tobacco mosaic virus coat protein gene. *Science,* 232: 738- 743.

Rubin, J.L., Gaines, C. and Jansen, R.A. 1984. Glyphosate inhibition of 5-enolpyruvylshikimate-3-phosphate synthase from suspension-culture cells of *Nicotiana sylvestris. Plant Physiology,* 75: 839-845.

Sanford, J.C. and Johnston, S.A. 1985. The concept of parasite-derived resistance-deriving resistance genes from the parasite's own genome. *Journal of Theoretical Biology,* 113: 395-405.

Schell, J. and van Montagu, M. 1983. The Ti plasmids as natural and as practical vectors for plants. *Bio/Technology,* 1: 175-180.

Sequira, L. 1984. Cross protection and induced resistance: their potential for plant disease control. *Trends in Biotechnology,* 2: 25-29.

Shah, D.M., Horsch, R.B., Klee, H.J., Kishore, G.M., Winter, J.A., Tumer, N.E., Hironaka, C.M., Sanders, P.R., Gasser, C.S., Aykent, S., Siegel, N.R., Rogers, S.G. and Fraley, R.T. 1986. Engineering herbicide tolerance in transgenic plants, *Science,* 233: 478-481.

Steinrucken, H.C. and Amrhein, N. 1984. The herbicide glyphosate is a potent inhibitor of 5-enolpyruvylshikimic acid-3-phosphate synthase. *Biochemical and Biophysical Research Communications,* 94: 1207-1212.

Tumer, N.E., O'Connell, K.M., Nelson, R.S., Sanders, P.R., Beachy, R.N., Fraley, R.T. and Shah, D.M. 1987. Expression of alfalfa mosaic virus coat protein gene confers cross-protection in transgenic tobacco and tomato plants. *EMBO J.,* 6: 1181-1188.

Van den Elzen, P., Townsend, J., Lee, K. and Bedbrook, J. 1985. A chimeric hygromycin resistance gene as a selectable marker in plant cells. *Plant Mol. Biol.,* 5: 299-302.

Watrud, L.S., Perlak, F.J., Tran, M.T., Kusano, K., Mayer, E.J., Miller-Wideman, M.A., Obukowicz, M.G., Nelson, D.R., Kreitinger, J.P. and Kaufman, R.J. 1985. Cloning of the *Bacillus thuringiensis* subsp. *kurstaki* delta endotoxin gene into *Pseudomonas fluorescens:* Molecular biology and ecology of an engineered microbial pesticide. In: "Engineered Organisms in the Environment," H.O. Halvorson, D. Pramer, M. Rogul, Eds., American Society for Microbiology, Washington, pp. 40-46.

Use of Hormones and Ovule and Embryo Culture to Enhance Wide Crosses in *Arachis*

J.P. Moss, D.C. Sastri, and L. Aruna
ICRISAT
Patancheru, India

Introduction

A plant species maintains its identity by allowing gene exchange between members of the species, but preventing gene exchange with members of other species. Similarities between closely related species decrease with more distantly related species or genera, and at the same time there is usually a decrease in the ease of producing hybrids and the meiotic regularity and fertility of any hybrids that can be produced. Although breeders have relied almost exclusively on intraspecific gene transfer, and on meiotic recombination in the F_1 hybrids, to produce the vast majority of cultivated plants, many of these wild species are resistant to pests and diseases, and form a valuable gene pool.

The wild relatives of crop plants contain a wealth of desirable characters, not only resistance to stresses such as diseases, pests or inclement conditions, but also desirable growth attributes (Frey, 1983). Some wild *Arachis* species are resistant to important yield-reducing pests and diseases, and a few species contain multiple resistance (Stalker and Moss, 1987). Often these species are isolated from crop plants by mechanisms that are effective in nature, but can be overcome by various techniques. The resulting F_1 hybrids may be crossable with the cultivated species, or further manipulations may be necessary to produce segregating F_2 populations, but the resulting derivatives can be very valuable, either as potential cultivars, or as new germplasm (Moss, 1985).

The natural barriers to interspecific hybridization that have evolved to maintain the identity of a species can be classified into prefertilization and postfertilization. The barriers may be single and simple, controlled by a simple gene system for which variation exists in the form of crossable species that can be easily exploited, or may be complex, as there are many processes involved in sexual reproduction. These include pollen recognition, pollen germination, pollen tube penetration, pollen tube growth through the style, penetration of the micropyle, fertilization, endosperm development, embryo development, fruit development, and seed maturation. The chemistry and morphology of the flower, pollen, stigma, style, fruit, and seed differ between genera, and truly wide sexual hybrids may

always fail due to these differences. A range of techniques have been reported as effective in overcoming barriers to hybridization.

The *Arachis* flower is a modified legume flower, where the calyx, corolla, and anthers are borne at the top of an elongated tubular hypanthium, at the base of which is the ovary. The style extends the full length of the hypanthium tube, and the stigma is positioned between the anthers. After fertilization, a meristem is formed at the base of the ovary, and a gynophore or "peg" grows into the soil, and the pod is formed under the soil. Geotropic response and elongation of the peg is dependent on the presence of the meristem, and is not dependent on the presence of the ovary (Ziv and Zamsky, 1975).

Interspecific crosses are of interest to the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), as certain wild species contain resistances to important pests and diseases (Moss, 1985) for some of which resistance is not available in cultivated groundnut. A number of derivatives have been produced from interspecific crosses and used by breeders, but genes from some species have not yet been introgressed into the cultivated groundnut.

The genus *Arachis* has been classified into seven sections (Gregory and Gregory, 1979). Section *Arachis* comprises tetraploid *A. hypogaea*, the cultivated groundnut or peanut, and one tetraploid and a number of diploid wild species. Section Rhizomatosae comprises a diploid species, and one or more tetraploid species, which are rhizomatous perennials. These are resistant to many pests and diseases (Moss, 1988; ICRISAT, 1987), and production of hybrids has a high priority in the ICRISAT Cytogenetics Unit. Use of mentor pollen, and mentor pollen leachate, with or without hormones, showed that hormone application at the time of pollination was an effective means of stimulating hybrid peg formation (Sastri and Moss, 1982).

This paper describes techniques whereby hybrid embryos were routinely produced and stimulated to develop to the stage where they could be cultured, either to produce callus for regeneration of hybrid shoots, or to produce seedlings.

Materials and Methods

The species and accessions used in the main study are listed in Table 1. Other species are listed in Table 3. *A. hypogaea* was used as female parent, as *Rhizomatosae* species produce few seeds. Female plants were emasculated in the evening and pollinated the

following morning, the standard technique for groundnut (Norden, 1980). Hormones used to induce peg and pod production were kinetin (6-furfurylaminopurine) (Kn), indole-3-yl acetic acid (IAA), 1-naphthyl acetic acid (NAA), 2,4-dichlorophenoxy acetic acid (2,4-D), and gibberellic acid (GA). Kn, NAA, IAA, 6-benzyl amino purine (BA) and indole butyric acid (IBA) were used in culture media.

Table 1. Identity and Source of Taxa Used in Crossing Program.

Female parents
Section *Arachis*
Arachis hypogaea
ssp. *hypogaea* var. *hypogaea*
cv *M 13 (Punjab Agricultural University, Ludhiana, India)*
cv *MKI 374 (Kadiri, India)*
cv *Robut 33-1 (Kadiri, India)*
ssp. *fastigiata* var. *vulgaris*
cv TMV 2 (Tinivandum, India)
Male parent
Section *Rhizomatosae*
Arachis sp. PI 276233[1] (Reading University, U.K.)[2]

[1]PI = USDA Plant Inventory Number.
[2]Original collection from Paraguay.

Hormones were applied at the time of pollination by immersing cotton wool in aqueous hormone solutions, and wrapping the wetted cotton wool around the base of the hypanthial tube. Hormones were applied to the peg by wrapping hormone-wetted cotton wool around the peg at the soil surface, or applying hormones in lanolin to the base of the peg.

Sixty days after pollination, pegs or immature pods were collected, surface sterilized, and ovules excised. Ovules were cultured on filter paper bridges over liquid MS medium (Murashige & Skoog, 1962) with 3% sucrose, 0.1 mg L^{-1} Kn and 0.075 mg L^{-1} IAA until embryos had grown out of, or could be excised from, the ovules. Heart-shaped or early cotyledonary embryos were cultured on MS medium with 3% sucrose, 0.01 mg L^{-1} NAA and 0.1 mg L^{-1} BA to stimulate further growth into seedlings, but the less-developed globular embryos were cultured on MS with 3% sucrose, 2.0 mg L^{-1} NAA and 0.5 mg L^{-1} BA to simulate callus formation and subse-

quent shoot production. Shoots were rooted on filter paper supports in liquid MS with 3% sucrose, 2 mg L^{-1} NAA and 1 mg L^{-1} IBA, and rooted shoots transferred to soil in pots. Unrooted shoots were excised from callus and grafted onto parental stocks.

Results

Effects of Hormones on Peg Production

The effect of five hormones (Kn, IAA, NAA, 2,4-D, and GA) on peg production was tested (Table 2).

Table 2. Effect of Hormones on Peg and Pod Production When *Arachis hypogaea* is Pollinated with *A.* sp. 276233.

Treatment	No. of Pollinations	Pegs/ Pollination (%)	Pods/ Peg (%)
Control	85	15	0
Kn 0.02 ppm	57	44	68
Kn 0.2 ppm	89	23	50
Kn 2.0 ppm	28	36	90
IAA 10 ppm	88	9	25
25 ppm	79	19	0
50 ppm	81	5	0
NAA 10 ppm	52	19	80
25 ppm	95	33	45
50 ppm	27	4	0
2,4-D 25 ppm	54	11	0
GA 25 ppm	144	56	20

Control (water) resulted in 15% peg production. Kn and GA were consistently better at all concentrations tested, and GA at 44 ppm stimulated the most pegs (86%). Although NAA at 25 ppm gave 33% pegs, other concentrations and the other auxins tested, IAA and 2,4-D, did not increase peg production appreciably, or produced fewer pegs than the controls.

Effect of GA on Other Interspecific Crosses in Arachis

GA treatment was used in a range of interspecific and intersectional crosses in *Arachis*, which do not usually produce pegs or pods (Table 3). Section *Triseminale* (2n = 20) has remained isolated from the rest of the genus (Gregory & Gregory) but produced 79% pegs, 39% of which formed pods, when crossed with *A. duranensis*

Table 3. Peg and Pod Production after Gibberellin Treatment in Some Intersectional Crosses.

	Pollinations (no.)	Pegs/Poll (%)	Pods/Peg (%)
Section *Arachis* x Section *Triseminale*			
A. duranensis (2n = 20) x *A. pusilla* (2n = 20)	33	79	39
A. hypogaea cv Robut 33-1 x *A. pusilla* (2n = 20)	78	46	0
Section *Arachis* x Section *Erectoides*			
A. hypogaea cv Robut 33-1 x *A. rigonii* (2n = 20)	45	64	13
A. hypogaea cv M 13 x *A. rigonii*	18	94	22
A. hypogaea cv TMV2 x *A. rigonii*	43	86	26
Section *Arachis* x Section *Extranervosae*			
A. hypogaea cv Robut 33-1 x *A. villosulicarpa* (2n = 20)	39	59	3
A. hypogaea cv MK 374 x *A. villosulicarpa* (2n = 20)	9	89	11
Section *Extranervosae* x Section *Triseminale*			
A. villosulicarpa (2n = 20) x *A. pusilla* (2n = 20)	24	54	46
Section *Arachis* x Section *Rhizomatosae*			
A. hypogaea cv Robut 33-1 x *Arachis* sp. Coll. 9649	82	44	6
A. hypogaea cv Robut 33-1 x *Arachis* sp. Coll. 9797	46	57	2
A. hypogaea cv Robut 33-1 x *Arachis* sp. Coll. 9806	26	62	0
A. hypogaea cv Robut 33-1 x *Arachis* sp. Coll. 9813	7	30	50
A. hypogaea cv TMV2 x *Arachis* sp. Coll. 9649	11	73	0
A. hypogaea cv MK 374 x *Arachis* sp. Coll. 9649	26	42	15
A. hypogaea cv MK 374 x *Arachis* sp. Coll. 9797	14	65	0
A. hypogaea cv M 13 x *Arachis* sp. PI 276233	75	56	5
A. hypogaea cv Chico x *Arachis* sp. PI 276233	58	66	9
A. hypogaea cv Chico x *Arachis* sp. PI 9649	26	73	19

(2n = 20). Peg production was lower in the cross with *A. hypogaea*, and no pods were formed. *A. hypogaea* produced pegs and pods when pollinated with species from sections *Erectoides and Extranervosae*.

Pod Production

Pegs that have been produced following GA treatment at the time of pollination either failed to reach the soil, or failed to produce pods.

Four cultivars, MK 374, Robut 33-1, M 13, and TMV 2 were pollinated by *A.* sp. 276233. GA was applied at pollination, and 10, 25, 50 or 100 ppm IAA was applied in lanolin to the developing peg 10, 15, 20, or 25 days after pollination (Table 4). The percentage pod formation without IAA treatment ranged from 10 (M 13) to 37 (TMV 2). After IAA treatment, percentage pod formation ranged from 0 (M 13) to 83 (TMV 2). IAA increased the number of pods formed in all cultivars. IAA had little overall effect in Robut 33-1, but in the other cultivars, mean pod production at any one time of application or at any concentration was higher than the controls. There was no significant difference between the concentrations used, though the highest pod production was usually after treatment with 50 or 100 ppm IAA. The difference between times of application was more marked, in most cases 20 or 25 days after pollination being the best time.

The effect of other phytohormones on production of pods on GA-induced pegs was also tested. Kinetin at 0.02 ppm applied to Robut 33-1 10 days after pollination increased pod production, but other times of application were not as good as with IAA. Similar results were obtained using MK 374. Kinetin applied to TMV 2 did not increase pod formation.

NAA was applied to pegs induced by GA on Robut 33-1 pollinated with A. sp. 276233 (Table 5). Results were better than when Robut 33-1 was treated with IAA.

Sequential Hormone Treatments to GA-induced Pegs

Two or three applications of IAA or Kn were compared with single treatments (Table 6). IAA applied 10 and 17 days after pollination was better than a single treatment at 10 days, but all the other multiple treatments decreased the number of pods produced.

Table 4. Frequency of Pod Formation on GA-induced Pegs of *A. hypogaea* x *A.* sp. 276233 after Treatment with Different Concentrations of IAA in Lanolin.

A. hypogaea Cultivar	IAA Conc (ppm)	Pods/Peg (%)				
		Time of Application (Days After Pollination)				
		10	15	20	25	Mean
MK 374	Control[1] = 20					
	10	42	43	67	48	48
	25	53	39	69	32	44
	50	25	32	56	34	36
	100	49	37	38	51	43
	Mean	43	38	56	41	
Robut 33-1	Control = 23					
	10	10	14	18	9	13
	25	27	9	20	14	17
	50	14	13	16	27	16
	100	31	18	8	40	20
	Mean	20	13	17	15	
M13	Control = 10					
	10	13	31	16	31	23
	25	8	33	27	6	20
	50	NA[2]	27	35	22	29
	100	(0)[3]	7	43	50	36
	Mean	11	26	32	25	
TMV 2	Control = 37					
	10	40	55	48	76	52
	25	39	53	65	47	50
	50	21	83	56	81	61
	100	85	31	69	69	57
	Mean	43	51	59	68	
Grand Mean		32	30	37	35	

[1]Control = GA treatment at pollination.
[2]NA = Not Attempted.
[3]( ) = less than 10 pollinations.

Ovule and Embryo Sizes

Very few of the pods induced by IAA or NAA on GA-induced pegs after cross pollination matured, and such pods did not contain viable seeds. Immature pods were harvested, but embryos were too small to dissect and culture. However, ovules could be excised and cultured.

Table 5. Frequency of Pod Formation on GA-induced Pegs on Robut 33-1 Pollinated with *A.* sp. 276233 after Treatment with NAA in Lanolin.

NAA Conc (ppm)	Pods/Peg (%)			
	Time of Application (Days after Pollination)			
	10	15	20	25
Control = 24				
10	48	44	37	42
25	61	41	35	35
50	12	33	22	77
100	39	52	40	30

Table 6. Effect on Sequential Applications of Hormones on Pod Production on GA-induced Pegs in Robut 33-1 x *A.* sp. 276233.

Hormone	Pods/Peg (%)						
	Time of Application (Days after Pollination)						
1st Application	10	15	20	10	10	15	15
2nd Application				17	17	22	22
3rd Application					24		29
Control[1] = 19							
IAA (25 ppm)	27	11	16	32	3	6	8
Kn (0.02 ppm)	50	19	24	8	15	0	5

[1]Control = GA treatment at pollination.

A. hypogaea was crossed with *A.* sp. 276233, treated with GA, and developing pegs treated with different concentrations of IAA 10, 15, 20 or 25 days after pollination. The number of pods produced was consistent with other results; and ovules and embryos were excised 60 days after pollination and measured (Table 7). Ovule size was increased by some of the treatments, the best being 25 ppm 20 days after pollination. Concentrations 25 ppm or higher applied within 20 days after pollination were most effective. Embryo size was increased by all treatments. Although these embryos could be dissected from the ovules and cultured, culture of whole ovules gave better overall success rates.

Ovule Culture

Ovules were dissected from developing pods 60 days after pollination and cultured on filter paper bridges over liquid MS medium

Table 7. Effect of IAA in Lanolin on Pod-set and Ovule and Embryo Development in Gibberellin-induced Pegs in *A. hypogaea* cv. Robut 33-1 x *Arachis* sp. PI 276233.

Concentration of IAA (ppm)	Time of Application (Days after Pollination)			
	10	15	20	25
	Pods/Peg (%)[1], Control = 16%			
10	21	32	29	16
25	14	14	27	19
50	15	21	30	33
	Ovule length (mm), Control = 2.3 mm			
10	2.9	2.1	3.1	1.9
25	3.2	2.3	3.5	2.5
50	2.5	3.3	2.5	2.6
	Embryo length (mm), Control = 0.27 mm			
10	1.5	0.6	0.6	
25	2.6	1.0	1.5	0.6
50	1.0	0.9	0.9	0.6

[1]Total pegs treated = 368; minimum number in any treatment = 22.

supplemented with Kn and IAA (Table 8). Lower concentrations of IAA were more effective, though there was little difference in the final numbers of embryos that grew, as from 6 to 9 percent of ovules produced embryos that grew when excised and cultured.

Table 8. Response of *A. hypogaea* TMV2 x *A.* sp. 276233 Hybrid Ovules and Embryos When Cultured on Filter Paper Bridges over MS Medium with 0.1 ppm Kn and Different Concentrations of IAA.

IAA Conc (ppm)	Number of				% Ovules Producing Viable Embryos
	Ovules		Embryos		
	Cultured	Growing	Cultured	Growing	
0.0	138	110(80)[1]	38	13(34)	9
0.1	54	29(54)	11	4(36)	7
0.2	275	149(54)	47	16(34)	6
0.5	72	31(43)	24	6(25)	8

[1]Figures in parentheses are percentages.

Embryo Culture

Ovules were cultured on filter paper bridges over MS medium with 3% sucrose, 0.1 ppm Kn, and 0.075 ppm IAA. After one month of ovule culture, embryos were dissected from the ovules and cultured on a range of media to induce callus formation or to stimulate normal embryo growth and germination. However, the latter was only possible with heart-shaped and early cotyledonary embryos. On the

media used, MS + 3 or 5% sucrose, with either 2.0 mg L^{-1} NAA and 0.5 mg L^{-1} or 0.5 mg L^{-1} NAA and 0.5 mg L^{-1} BA, all the later stage embryos grew or formed callus, but not all the globular or late globular embryos responded (Table 9).

Table 9. Response of Hybrid Embryos Cultured at Different Stages of Development.

Stage of Development of Embryo							
Globular		Late Globular		Heart		Early Cotyledonary	
NC	NG	NC	NG	NC	NG	NC	NG
A. hypogaea cv M13 x *A.* sp. 276233							
6	1(17)	29	6(21)	1	1(100)	5	5(100)
A. hypogaea cv MK 374 x *A.* sp. 276233							
23	4(17)	61	29(48)	1	1(100)	6	6(100)

NC = Number cultured; NG = Number which grew; ( ) = Percentage.

Embryos were therefore segregated according to stage of growth, and developed embryos were cultured on MS with 3% sucrose, 0.01 mg L^{-1} NAA, and 0.1 mg L^{-1} BA. These embryos grew normally. Less-developed embryos were cultured on the same basic medium but with 2.0 mg L^{-1} NAA and 0.5 mg L^{-1} BA to induce callus formation. Callus was subcultured on the same medium, and multiplied to produce large amounts of callus from one cross-pollination. Callus subsequently transferred to MS with 0.5 mg L^{-1} NAA and 0.5 mg L^{-1} BA formed shoot buds. Cultures with shoot buds were transferred to MS with 0.1 mg L^{-1} NAA and 1.0 mg L^{-1} BA, and shoot buds elongated. The resultant shoots were either grafted onto stocks of *A. hypogaea* or *A.* sp. 276233, or supported on filter paper over MS with 2.0 mg L^{-1} NAA and 1.0 mg L^{-1} IBA to induce root formation.

Discussion

Treating flowers with GA at the time of pollination was a simple and effective way to increase the frequency of hybrid pegs. Other techniques such as mentor pollen were also effective but were not adopted as a routine treatment, as GA application using the cotton wool method is quick, does not need special skills, apparatus or the care needed in producing mentor pollen or mentor pollen leachate, and can be done by anyone who can emasculate and pollinate groundnuts without appreciably decreasing the number of pollinations. The levels of peg production achieved are entirely

satisfactory for a practical interspecific crossing program, and are of the same order as in intraspecific crosses.

The mode of action of GA in promoting hybrid peg production in *Arachis* is not clear. Peg growth is the result of the formation of a meristem basal to the ovules, and this meristem is not active in hybrid pegs. At the given concentrations GA does not stimulate peg formation unless fertilization has taken place, as unpollinated ovaries do not form pegs when treated with GA; the maximum peg formation achieved was similar to the maximum achieved with intraspecific crosses, and probably relates to the maximum rate of fertilization that is commensurate with the damage to the style during hand emasculation and pollination. GA has been applied as a spray, lanolin paste, or injection to developing fruits to prevent fruit loss in wide crosses in a number of genera (de Nettancourt, 1977). There is no zone of abscission and no comparable shedding of fruit in *Arachis* crosses. The only abscission zone is at the base of the hypanthium and is involved in the shedding of the flower (Pattee and Mohapatra, 1986). This zone is adjacent to where the meristem develops, and it is possible that GA provides a stimulus to overcome the quiescence of the meristems that is lacking in hybrids.

The auxins IAA and NAA were the most effective of the hormones tested for pod formation, though Kn also had an effect. IAA applied in lanolin, at either 50 or 100 ppm, 20 or 25 days after pollination was the most effective treatment, although there were differences between the four genotypes used.

A single treatment with auxin was equal to or better than multiple treatments in the *Arachis* crosses tested, though in other intersectional or intergeneric crosses multiple treatments, or mixtures of hormones (Attman, 1988), may be needed to maintain fruit development. The action of auxin may be to inhibit further peg elongation, as occurs in *A. hypogaea*, and to initiate pod formation, which is normally initiated by auxins produced by the ovary (Jacobs, 1951).

Enhancement of peg and pod production by hormone treatments did not result in hybrid seed. The pegs and pods grew slowly, and when harvested 60 days after pollination, they contained immature ovules at a time when pods resulting from self-pollination of the maternal parent would normally have been fully mature. In addition to the fact that the hybrid tissue grew very slowly, the growth of the maternal tissue was also much slower than it would have been after selfing.

Even when the heart-shaped or early cotyledonary embryos had been isolated from the maternal tissue and cultured on a range of media, growth was still slow, and the seedlings grew slowly when transferred to soil. Some of the callus which was produced grew rapidly, and the frequency of shoot formation on regenerative callus was comparable to that on similar *A. hypogaea* callus. Grafts however, did not produce flowers, and only 17 percent of grafts survived for more than 4 months (ICRISAT, 1987), though many other interspecific hybrids in *Arachis* are vegetatively vigorous and flower freely. Flowers were produced by a hybrid plant similar to those described in this paper, except that they were derived from a seed which did mature from a hormone-aided cross between *A. monticola* (a close wild relative considered by some to be a subspecies of *A. hypogaea*) and *A.* sp. 276233.

Conclusions

The need for two hormone treatments to maintain pod growth, and for ovule and embryo culture to maintain the growth of the hybrid zygote, along with the slow growth and lack of flowering of the hybrid plant, indicate that section *Rhizomatosae* has diverged considerably from *A. hypogaea*. The barrier to hybridization apparently is not a simple one. However, the production of hybrids indicates that there is potential for introgression from *Rhizomatosae*, and the techniques can be used to produce hybrids from other intersectional crosses.

References

Altman, D.W. 1968. Exogenous hormone applications at pollination for *in vitro* and *in vivo* production of cotton interspecific hybrids. Plant Cell Reports (in press).

Frey, K.J. 1983. Genes from wild relatives for improving plants. pp. 1-20 (Eds. Yap, T.C., Graham, K.M., and Jalani Sukaimi) *In:* Proceedings of the Fourth International SABRAO Congress—Crop Improvement Research. Published by The Society for the Advancement of Breeding Researches in Asia and Oceania.

Gregory, M.P. and Gregory, W.C. 1979. Exotic germplasm of *Arachis* L. interspecific hybrids. J. Hered. 70, 185-93.

ICRISAT (International Crops Research Institute for the Semi-Arid Tropics). 1987. ICRISAT Annual Report 1986. Patancheru, A.P. 502 324, India: ICRISAT.

Moss, J.P. 1985. Wild species in crop improvement. *In:* Proc. Inter-Center Seminar on IARCs and Biotechnology. pp. 199-208 Laguna, Philippines. International Rice Research Institute.

Moss, J.P., Rao, V.R.R., and Gibbons, R.W. 1988. Evaluating the germplasm of groundnut (*Arachis hypogaea*) and wild *Arachis* species at ICRISAT. *In:* The use of crop genetic resources collections, eds. Brown A.H.D. Frankel, O.H. Marshall D.E. and Williams J.T. Camb. Univ. Press Cambridge (in press).

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia P1. 15, 473-93.

Nettancourt, D. de. 1977. Incompatibility in Angiosperms. Springer, Berlin.

Norden, A.J. 1980. Peanut. *In:* Hybridization of Crop Plants. American Society of Agronomy—Crop Science Society of America, 667 S. Segoe Rd., Madison, WI 53711.

Pattee, H.E. and Mohhapatra, S.C. 1986. Hypanthium and style senescence in relation to ovary development in Arachis hypogaea L. Botanical Gazette 147(3):302-311.

Sastri, D.C. and Moss, J.P. 1982. Effects of Growth Regulators on Incompatible Crosses in the Genus *Arachis* L. Journal of Experimental Botany 33(137):1293-1301.

Stalker, H.T. and Moss, J.P. 1987. Speciation, Cytogenetics, and Utilization of *Arachis* species. Advances in Agronomy, 41:1-40.

Ziv, M. and Zamski, E. 1975. Geotropic responses and pod development in gynophore explants of peanut (*Arachis hypogaea* L.) cultured in vitro Ann. Bot. 39, 579-83.

Alien Germplasm for Wheat (*Triticum Aestivum* L.) Improvement Facilitated By Cytogenetic Manipulation and Use of Novel Techniques

Abul-Mujeeb-Kazi and Robert Asiedu
CIMMYT, Wheat Program
Mexico D.F.

Introduction

There are approximately 250 perennials amongst the 325 Triticeae species (Dewey, 1984), and relatively few of these have been hybridized with wheat. Those that have been are predominantly included in the *Thinopyrum* group. Over the last decade and a half, phenomenal success has been achieved in the production of complex hybrids amongst the Triticeae, leading to a potential stock of valuable alien genetic material introduced from wide hybrids. Wide hybrids provide cytological data, evolutionary or phylogenetic information about the parental species, and the practical motivation associated with the introduction of significant characteristics from the alien species for wheat improvement. These alien transfers can diversify variability for both biotic and abiotic situations, two aspects that are of considerable functional difference. In one case, the pathogenic system is vulnerable to mutational events leading to breakdown of resistance, whereas the other aspect deals with physiological traits devoid of mutational changes, being thus categorized as dynamic and static systems (Mujeeb-Kazi and Kimber, 1985). Recognizing the above and also the fact that variation can be incorporated into wheat via intervarietal crosses, alien genetic variability is additive to plant breeding efforts due to its unique origin. This is based upon diverse alien genome categorizations, a variability source that would otherwise be inaccessible to breeders adopting strict conventional plant improvement procedures.

Successes of usable alien genetic incorporation are not numerous, but they have certainly made their impact (Sharma and Gill, 1983a; Mujeeb-Kazi and Kimber, 1985) despite the fact that these achievements have primarily been for simply inherited genetic traits. Hence, objectives associated with selection within populations that permit genetic expressivity of simplistically heritable attributes rank high in exploitation of intergeneric alien gene transfers. The vulnerability of simple genes is quite obvious even when these are pyramided with other genes. Other attributes with ill-defined genetic information, circumstantially inferred to be controlled by polygenic recessive systems, have complex heritability

and may have more success via interspecific en-bloc gene transfers rather than staggered intergeneric exchanges. Some of these complex attributes are associated with resistances or tolerances to *Fusarium graminearum, Helminthosporium sativum, Neovossia indica,* salt, drought, and mineral deficiencies or toxicities.

Several complexities exist in effecting successful alien introgressions, and there are different methodologies of attaining wheat x alien species combinations. The alien species could be screened for specific resistances or tolerances and then be hybridized with wheat. Alternatively, the species could be first hybridized and lead to advanced derivative screening. Irrespective of the procedure adopted, hybrid production (intergeneric or interspecific) is the critical initial base from which additional manipulations may promote effective genetic transfers.

Germplasm

The alien species (annual or perennial) utilized in wide hybridization are of *Aegilops, Agropyron, Elymus, Haynaldia villosa, Hordeum vulgare, Heteranthelium, Henrardia, Secale cereale, aeniantherum, Triticum monococcum, T. boeticum, T. urartu, T. araraticum,* and *T. tauschii.* The species are characterized by genomic diversity or substantial similarity and are either monotypic or comprised of several hundred accessions.

Hybrid Production

Barriers to production of wide crosses occur at various stages in the ontogeny of the hybrid comprising of: (a) parental choice, (b) emasculation procedure, (c) prepollination treatment, (d) pollination process, (e) post-pollination treatments, and (f) embryo excision plus culture (Mujeeb-Kazi and Kimber, 1985). The most simple hybrids, it appears, were produced by the conventional wheat crossing procedures. Variations originated when distant crosses were attempted, leading to the adoption of an array of manipulative techniques. These enabled the (a) to (f) characterization of the salient hybrid production stages that are more a reflection of constraints for the intergeneric hybridization category rather than the interspecific combinations used in the program. The crossability barriers are less critical in the latter case, but where prevalent can be readily eliminated through incorporation of bud pollination and embryo rescue techniques. Plantlet processing after embryo differentiation in Murashige and Skoog (1962) or Taira and Larter (1978) media has been elaborated in reports of Mujeeb-Kazi and Rodriguez (1983a, 1983b). Hybrid identification procedures are morphological, cytological, or biochemical (Mujeeb-Kazi et al., 1987;

Asiedu and Mujeeb-Kazi, 1987). Their practical utilization is determined through genomic analysis made possible by the development of numerical methods of assessing genomic affinity (Kimber and Hulse, 1978; Kimber et al., 1981; Alonso and Kimber, 1981; Kimber and Alonso 1981; Espinasse and Kimber, 1981). Interpretations from the above methods enable application of pertinent techniques relative to alien transfers (Kimber, 1984). When the mean arm-pairing frequency and the relative affinity have values approaching one, the transfer can be made by recombination. At intermediate values of mean arm-pairing frequency, increased homologous pairing can be induced by changes in the systems regulating chromosome pairing. At very low values of the mean arm-pairing frequency, irrespective of the value of the relative affinity, irradiation or centric break-and-fusion in derived aneuploids is the optimum method (Kimber, 1984).

Alien Transfer Constraints and Some Solutions

In alien genetic transfers so far, only rather simply inherited traits have been introgressed (Dewey, 1984; Knott and Dvorak, 1976). Transferring polygenically controlled characters that are presumably recessive in nature appears too difficult to achieve via intergeneric hybridization if short-term projections are made. Where polygenes are located on more than one alien chromosome, each gene could be introduced into a separate wheat background prior to pyramiding these independently transferred genes into a single line or variety. We must not, however, preclude the possibility of major gene influences in a polygenic system that would bestow major resistant/tolerant influences in a simple genetic manner and yield variable segregating patterns.

Despite the complexity of polygenic systems, solutions do exist that could facilitate gene transfer methodology. These are associated with:

(a) Chromosome 5B mechanism. There is considerable merit in attempting to enhance recombinations in F_1 hybrids by incorporating the *Ph* system (Darvey, 1984; Forster and Miller 1985; Mujeeb-Kazi et al., 1984, 1987; Sharma and Gill, 1983a, 1983b, 1983c). The procedures involve the use as maternal parents of (i) nulli-5B tetra 5A or 5D wheat stocks, (ii) mono- 5B, or (iii) *PhPh* mutant, etc., in the production of F_1 hybrids. Such F_1 hybrids would exhibit a high meiotic chromosome pairing frequency, thereby promoting incorporation of alien transfers in ideal locations within the wheat genomes.

(b) Partial or complete synthetic genomes. In the alien species, several are autotetraploids (*A.* or *P. stipaefolium*), segmental allotetraploids, or partial autopolyploids (*A.* or *T. curvifolium*, *A.* or *T. junceiforme*, *A.* or *T. scirpeum*), segmental allohexaploids (*A.* or *T. junceum*), or segmental autoallohexaploids (*A.* or *T. podperae*, *A.* or *T. varnense*) where there are two closely related genomes and the third genome is distinctly different from the other two.

In segmental allotetraploids or partial autopolyploids, repeated selfings of the backcross I self-fertile derivatives could potentially lead to "complete" synthetic genome formation (Mujeeb-Kazi and Miranda, 1984) (Figure 1). In segmental autoallohexaploids, there is the likelihood of the unrelated third genome to be eliminated and of fertile backcross I derivatives leading to formation of stabilized 'partial' synthetic genomes (Figure 2). The selfing is expected to promote reorganization of complex polygenes as a consequence of recombination.

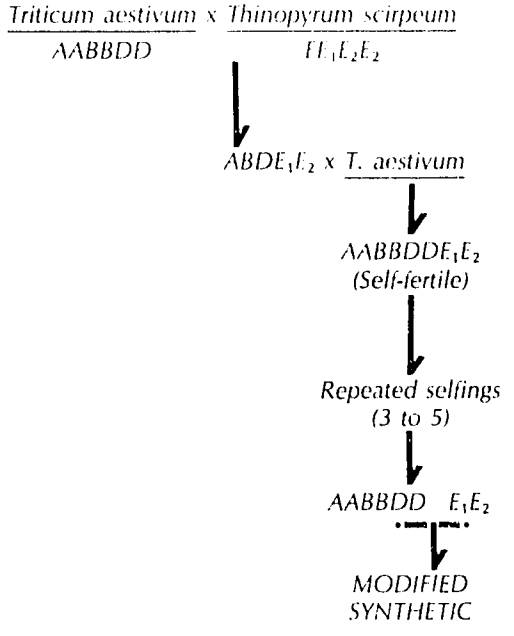


Figure 1. Development of a complete synthetic genome from hybridization of Triticum aestivum *x* Thinopyrum scirpeum *via Backcross I production and its repeated selfings to enhance E_1E_2 genomic reorganization.*

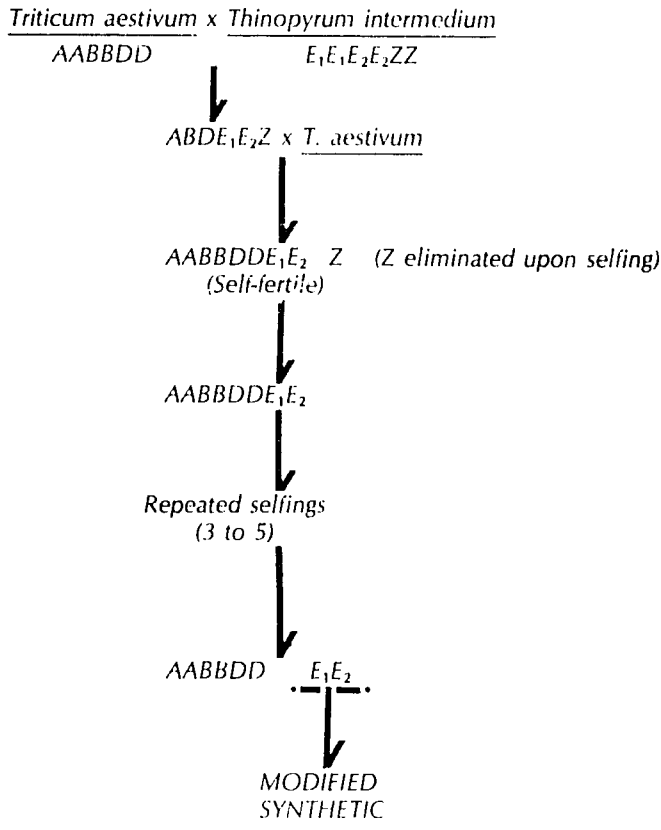


Figure 2. Development of a partial synthetic genome from hybridization of Triticum aestivum *x* Thinopyrum intermedium *via backcross I production and its repeated selfings to enhance E_1E_2 genomic reorganization with elimination of the unrelated Z genome.*

(c) Translocations have contributed significantly to disease resistance transfers (see review by Sharma and Gill, 1983a). Presumably, the major impact so far has been from the 1A/1R and 1B/1R translocations, greater for the 1B/1R (Rajaram et al., 1983). Other translocations of practical interest are the 5A/5R for copper efficiency and the probable utilization of 6RL rye arm for cereal cyst nematode resistance. In intergeneric hybridization, the advance of the F_1 hybrid to BCI via unreduced egg formation is a potent manipulative source of wheat-wheat, wheat-alien, or alien-alien exchanges (Mujeeb-Kazi et al., 1987). Numerous translocations have been reported in triticale-wheat crosses (Lukaszweski and Gustafson, 1982),

and this additionally supports the significance of translocations for practical applications, particularly wheat x rye (Asiedu and Mujeeb-Kazi, 1987). Ter Kuile et al. (1987) have proposed a scheme for inducing general or specific wheat D genome x alien chromosome-induced translocations. The general translocation induction process involves crossing of the *T. aestivum* x alien F_1 hybrid with *T. turgidum* that produces univalency for the entire wheat D and the alien genome chromosomes at meiosis (Figure 3). Specific alien translocations involve crossing a specifically desired disomic alien addition line that is *T. aestivum* based by *T. turgidum*. This would promote univalency at meiosis for the single alien addition chromosome and the entire *T. aestivum* D genome chromosomes, facilitating controlled centric-break-fusion between the entire D genome and the alien univalent (Figure 4).

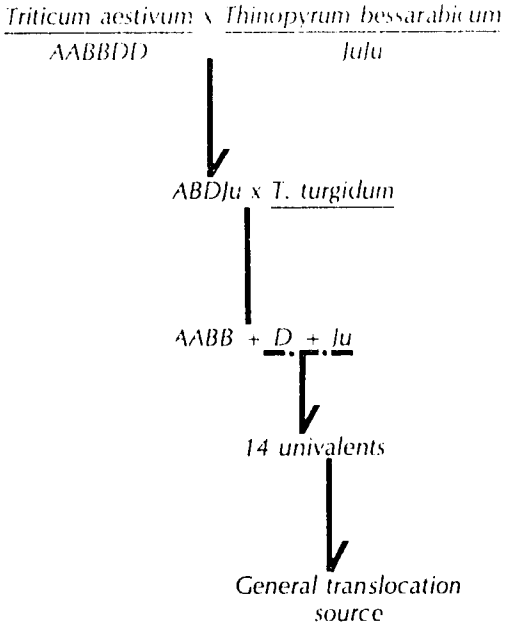


Figure 3. The general translocation induction process that utilizes 14 univalents (7 each) of the wheat D and T. bessarabicum *Ju genomes as a consequence of centric-break-fusion event/s.*

(d) Interspecific hybridization. Though rather simplistic as compared to intergeneric hybridization, it has the theoretical methodology advantage of yielding rapid practical returns over relatively short-term durations. The above concept is based on the following salient aspects: (i) ease of hybridization, (ii) field

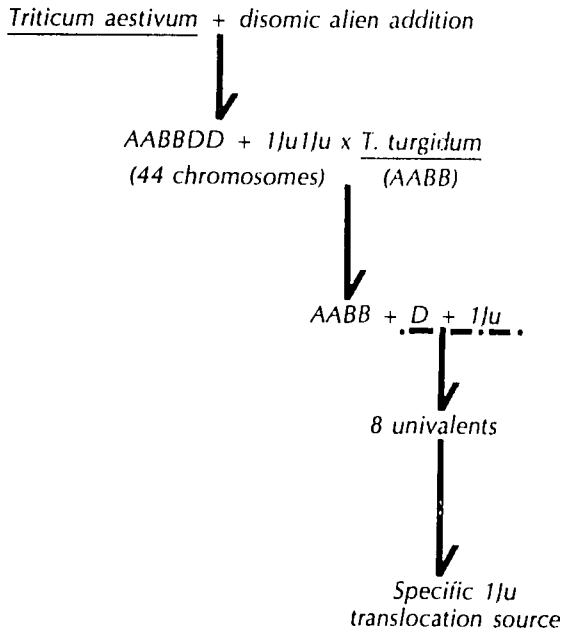


Figure 4. Specific translocation of a desired disomic alien addition and D genome of wheat involving 8 univalents (7 of D genome + 1 of the alien species).

oriented research, (iii) genomic similarity of the species with durum and bread wheat genomes, and (iv) as a consequence of high recombination the potential of en-bloc recessive polygenic transfers. There is tremendous flexibility of genomic manipulation approaches, and those with *T. tauschii* (2n = 2x = 14, DD) are represented in Figure 5 for developing *T. turgidum* x *T tauschii* synthetics, in Figure 6 for developing AABBDD synthetics by crossing extracted AABB from elite *T. aestivum* cultivars with *T. tauschii,* and in Figure 7 for improving elite *T. aestivum* cultivars by direct crossing with *T. tauschii* accessions. There are constraints pertaining to lack of expression of D genome characteristics but the range of variability in various species is so diverse that the advantages definitely warrant a thorough investigation. The above schematics of genome manipulation and utilization can be extended to the other genome sets.

Supporting Systems

In essence, the brief consideration of the gene transfer methodology presented under the above categories provides reasonable insight

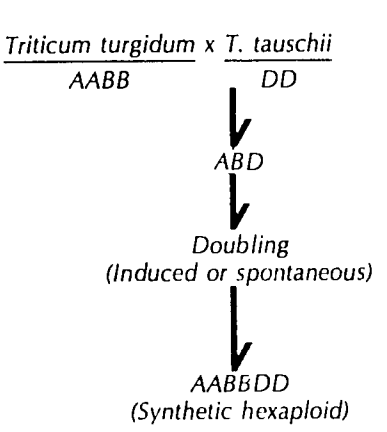


Figure 5. Derivation of synthetic hexaploids (AABBDD) from Triticum turgidum x T. tauschii *crosses.*

into the manipulative flexibility of the wheat cytogenetic system for facilitating alien genetic transfers. There are additionally several unique supporting systems that can extend the research potential of *Triticum*. These are all complementary facets for a coordinated approach at effecting alien transfers and a few are mentioned:

(a) Embryo and callus culture. Despite phenomenal successes in embryo culture, there is still ample need to manipulate embryo culture media and thus enable more distant hybrid embryos to differentiate. Embryo culture- based callus induction and plant regeneration has been studied with keen interest worldwide. More recently (TCCP, 1987), several *T. aestivum* and *T. turgidum* cultivars have been demonstrated to possess superb long-term callusing and regeneration capabilities. Cytological variation is a correlated aspect with regenerated plant development. The observed variation in advanced generations integrates with segregation of simply or polygenically inherited traits, and appropriate selections can be made. We do not feel, however, that callus-induced variation would be any different in nature as to that obtained in mutation breeding programs during M_2 to M_4 macro- and micromutation observational stages. In vitro screening has a decided advantage for the Triticeae that may include salt, aluminum, *Helminthosphorium* sp., fusaric acid, etc. The callus culture methodology further provides a means of promoting changes and/or alien genetic transfers (Lapitan et al., 1984, 1986, 1988). This approach is being applied to other wheat x alien F_1 hybrids that normally are poor recombinations; *T. aestivum* x *Aegilops variabilis* (Ter Kuile et al.,

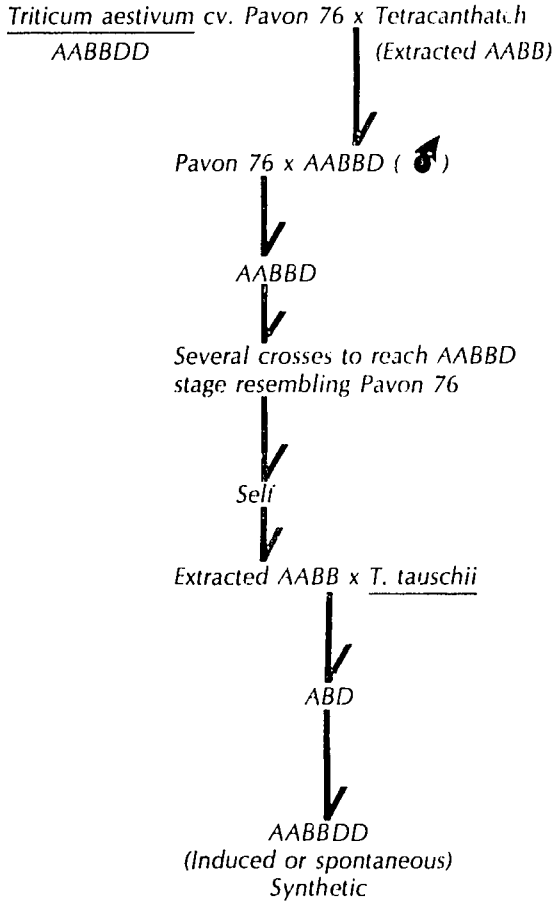


Figure 6. Extraction of AABB genomes from Triticum aestivum *L. cv. Pavon 76; hybrid with* T. tauschii *(DD) to produce the synthetic AABBDD.*

1987). More remarkable is the recent observation of chromosome number doubling in *T. aestivum* and *T. turgidum* x *Ae. variabilis* F_1 hybrids that have eluded conventional doubling attempts for approximately three decades. The doubling phenomenon needs further evaluation and warrants application towards other hard to double intergeneric combinations. The cytogenetic implications of callus-induced doubling are highly significant for controlled genetic transfers. A stable amphiploid is anticipated to eliminate aneuploidy that otherwise is rampant in backcross I derivatives (Jewell and Mujeeb-Kazi, 1982), thus eluding complete genetic evaluation.

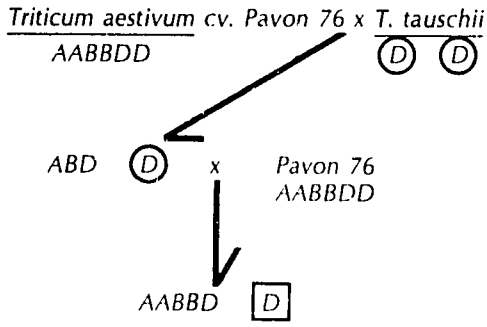


Figure 7. A mechanism for incorporation of DD genome variability into an elite Triticum aestivum *cultivar. Note that after 1 backcross only, 5/6 of the Pavon 76 genotype is restored.*

(b) Polyhaploid production. Presumably, the genetic system of polyhaploid generation in wheat based upon *T. aestivum* L. cv. Chinese Spring x *H. bulbosum* hybridization (Barclay, 1975) is superior both in terms of polyhaploid generation frequency and euploid formation upon doubling. The procedure also was extended to recover alien disomic additions (Islam and Shepherd, 1981; Islam et al., 1978) in wheat x barley crosses. Polyhaploids of wheat have also been reported from *T. aestivum* L. cv. Chinese Spring x *Zea mays* crosses in reasonably high frequencies (Laurie and Bennett, 1986). The anther culture-based wheat polyhaploid generation results are below the frequencies obtained in the above-mentioned examples, and when compared with this sexual cross study in a joint experiment (Inagaki et al., 1987), limitations of *T. aestivum* anther culture are obvious. Varietal responses abound in both approaches but apparently the *kr* genes on 5A, 5B and 5D are a major source of the high success of Chinese Spring in the sexual combinations.

Anther cultures or sexual processes of polyhaploid production in polyploid aliens have significant merit and warrant greater attention in order to better resolve the prevalent genomic relationships. This information will undoubtedly have tremendous impact on alien genetic transfer methodology.

(c) *Diagnostic markers and their significance.* A number of heritable characteristics have been associated with specific genomes, chromosomes, chromosome segments or genes, and these serve as markers for the identification of alien chromatin in wheat. Some of the markers are also applicable during the initial screen of alien populations for maximum genetic

variability and avoidance of unnecessary duplication. They provide the information required to make the right choice of materials, methods, and population size necessary for efficient and precise transfer of alien genetic material into wheat and the characterization of the transferred segments. Specific genes of agronomic importance could be tagged more quickly and easily in a population once they have been linked to some of these markers. Other applications include aneuploid identification, genetic analysis, chromosome assays, hybrid confirmation, and establishment of alien-wheat chromosome homologies. Four major groups of markers have applications in wheat wide crosses—morphological, genetic, cytological, and biochemical. They all have their advantages and limitations so that combinations of two or more classes of markers for specific projects are not uncommon (Landry et al., 1987).

Generally, however, the most useful markers are those that show high levels of polymorphism, are rapid, can be applied to seeds or during seedling stages, have no deleterious pleiotropic effects, and are inherited in a codominant fashion.

Morphological markers

The loss of chromatin from wheat or its gain from an alien source often results in modifications in plant morphology. Some of these changes have been associated with specific homologous groups (Miller and Reader, 1987) and thus serve as markers for those groups. Hairy peduncles of wheat-rye derivatives have long been associated with the presence of rye chromosome 5R (Chang, 1975).

Genetic Markers

Genes for resistance to a number of diseases and pests and tolerance to such environmental stresses like micronutrient toxicity or deficiency have been located on specific chromosomes of wheat and/or its relatives. For instance, resistance to leaf, stem, and yellow rusts serve as useful markers for rye chromosome 1RS (Koebner and Shepherd, 1986).

Cytological Markers

Direct identification of plant chromosomes using such features as chromosome size, arm ratio, possession of satellites, and/or secondary constrictions have been used either alone or in conjunction with other techniques (Endo and Gill, 1984; Fujigaki and Tsuchiya, 1985; Hsiao et al., 1986). Numerous stains have been used for this purpose including feulgen, aceto-carmine, and carbol fuchsin (Evans and Reed, 1981).

C-banding, which reflects constitutive heterochromatin, has been very useful in the identification of chromosomes or their segments in plants and animals since its discovery by Pardue and Gall in 1970. The C-banding patterns of wheat cultivars have since been reported (Gill and Kimber, 1984; Iordansky et al., 1978a, 1978b; Seal, 1982; Seal and Bennett, 1982) as have those of the relatives of wheat (Vosa, 1984; Teoh and Hutchinson, 1983; Sybenga, 1983; Friebe et al., 1987). Chromosomes of wheat and its relatives have also been identified on the basis of their N-banding patterns (Gerlach, 1977; Jewell, 1979; Schlegel and Gill, 1984; Endo and Gill, 1984). The value of chromosome banding in identification of wheat and alien chromosomes involved in translocations has been demonstrated (Gill and Kimber, 1977; Jewell, 1979; Lukaszewski and Gustafson, 1983). While Giemsa has been most extensively used in banding, other dyes like Leishman's, Wright's (Seal, 1982), Hoechst 33258, and DAPI (Sarma and Natarajan, 1973; Schlegel and Gill, 1984) have been applied in differential staining of chromosomes of the *Triticeae*. These staining techniques enable more detailed meiotic analysis through positive identification of chromosome segments involved in synapsis (Naranjo and Lacadena, 1979; Giraldez and Orellana, 1979; Singh and Shepherd, 1984).

The introduction of DNA sequences as cytological markers through *in situ* hybridization (Gall and Pardue, 1969, John et al., 1969) has further expanded the capacity for chromosome identification (Rayburn and Gill, 1987a). While some of the chromosome staining patterns revealed by the technique are similar to those produced by conventional Giemsa C-banding (Hutchinson and Lonsdale, 1982; Teoh et al., 1983; Hutchinson, 1983) there are many reports of situations where *in situ* hybridization has proven either clearly advantageous or a valuable complement (Hutchinson et al., 1980; Miller et al., 1982; Hutchinson et al., 1982; Teoh et al., 1983; Lapitan et al., 1986; Rayburn and Gill, 1986, 1987b). Many early workers used probes labeled with radioactive nucleotides like ^{3}H or ^{125}I and detected hybridization by autoradiography (Gerlach and Peacock, 1980; Hutchinson and Lonsdale, 1982; Hutchinson et al., 1982) but the use of biotin-labeled probes and detection by immunological and staining techniques is now in vogue (Manuelidis et al., 1982; Rayburn and Gill, 1985, 1986, 1987a, 1987b; Lapitan et al., 1986; Gillam, 1987). The nonradioactive probes have the comparative advantage of safety, stability, and speed.

Biochemical Markers

Biochemical markers may be subdivided as protein and DNA markers. The former has been more extensively used in work on the *Triticeae* and is comprised both of isozyme and storage protein

markers. Hart and Gale (1987) have presented a recent summary of chromosomal locations of biochemical/molecular loci reported in hexaploid wheat cultivar Chinese Spring which shows the short arm of chromosome 2 as the only arm not thus marked. They (Hart and Gale, 1987) also tabulated chromosomal locations of orthologous loci in other species in the tribe *Triticeae*. More of such loci are being established for many other species of the tribe (Asiedu and Mujeeb-Kazi, 1987). The many advantages of these markers are the speed and applicability to seeds and early seedling stages of plant growth. Using the Glucose Phosphate Isomerase enzyme locus on wheat chromosome arm 1BS as a marker, the 15th, 18th, and 21st International Bread Wheat Screening Nurseries of CIMMYT were demonstrated to carry 41, 43, and 52 percent, respectively, of the 1BL/1RS translocation (unpublished). This involved the assaying of about 250 lines per day (at 2 to 4 seeds per line) by one person which is only a fraction of the time required using cytological methods.

DNA markers in the form of restriction fragment length polymorphisms (RFLPs) are relatively new within the *Triticeae*, but genetic maps of crops like maize, tomato, and lettuce have been constructed or are being updated with RFLPs as markers (Helentjaris et al., 1986; Landry et al., 1987). Conceptually, RFLPs have the potential to saturate the genetic maps of most crops with markers as they have the capacity to detect many differences or changes in the DNA sequence which may not result in detectable gene products or changes in morphology. A number of laboratories are developing probes suitable for or specific to genomes in the *Triticeae* (Appels, 1986). As more of these become available, there would be a great improvement in our capacity to identify smaller and smaller fragments of alien chromatin transferred into wheat.

Conclusions

Since the pioneering work of the late Anton Kruse (Kruse, 1969, 1973), advances in intergeneric hybridization in the Triticeae have been extensive (Sharma and Gill, 1983a; Mujeeb-Kazi and Kimber, 1985). Hybrid production technology is simplified, hybrid identification procedures are well-developed, and hybrid advance methodology is clarified, with gene transfer constraints adequately identified and partially overcome. The need for novel complementary areas and superb resolution of various diagnostic techniques appear to be well- documented. This consequently sets in appropriate balance for the Triticeae crops a wide cross alien gene transfer program that encompasses sophisticated current approaches without any retraction from the important rapid field application practical goals. Research objectives are carefully weighed and are

principally dependent upon practical returns. Collaborative research arrangements follow cautious evaluation as to their crop production duration significance, that should not be too basic a research function.

There seems to be no major impediment in wheat wide crosses to restrict genetic advances at the plant level. The germplasm that emanates as a consequence of cytogenetic manipulation forms the backbone for diagnostic technology applications to thrive upon and simultaneously enables the breeding component to forge ahead without crucial time lapses between the various developmental phases of the program. Collaborative research in areas of novel system applications and diagnostic procedures is, and will further become, a major incentive towards effective alien genetic transfer research understanding, productivity, and end-product dissemination at budget-efficient standards. The program perspectives of short-term (interspecific hybridization) and long-term (intergeneric hybridization) stretched over 7 to 12 years are anticipated to provide quality returns for the applied mandate of crop improvement via alien genetic variability incorporation.

The program structure has precise facets of further linking the plant level manipulation phase with cellular and molecular approaches, two aspects that are essential contributors to program functionality and effectiveness. A number of very desirable approaches are anticipated to subsequently emerge for the monocotyledonous plants, notably the Triticeae. When these research breakthroughs are functionally refined and applicable they undoubtedly will either find complementary usage in wheat improvement, or may possess the potential to totally replace several conventional stages of genetic manipulation. We are receptive to and cognizant of these futuristic changes and have not delved into any detail at this stage due to lack of adequately reported progress within the tribe Triticeae.

References

Alonso, L.C. and Kimber, G. 1981. The analysis of meiosis in hybrids. II. Triploid hybrids. Can. J. Genet. Cytol. 23:221-234.

Appels, R. 1986. Evolutionary change within chromosomes of the grasses and the development of probes for identifying specific chromosome segments. Annual Report (1985-86), CSIRO Division of Plant Industry, Canberra, Australia.

Asiedu, R. and Mujeeb-Kazi, A. 1987. Diagnostic Applications of Isozyme Markers in Wheat (*Triticum* spp.) Wide Crosses. Agronomy Abstracts pp. 54.

Barclay, I.R. 1975. High frequency of haploid production in wheat (*Triticum aestivum*) by chromosome elimination. Nature 256:410-411.

Chang, T.D. 1975. Mapping of the gene for hairy peduncle (*Hp*) on rye chromosome 5R. Can. J. Genet. Cytol. 17:127-128.

Darvey, N.L. 1984. Alien wheat bank. Genetics, 107:(Suppl.):24.

Dewey, D.R. 1984. The genomic system of classification as a guide to intergeneric hybridization with the perennial Triticeae. In: Gene manipulation in plant improvement, 16th Stadler Genetics Symposium. Edited by J.P. Gustafson. Plenum Press, New York. pp. 209-279.

Endo, T.R. and Gill, B.S. 1984. Somatic karyotype, heterochromatin distribution, and the nature of chromosome differentiation in Chromosoma 89:361-369.

Espinasse, A., and Kimber, G. 1981. The analysis of meiosis in hybrids IV. Pentaploid hybrids. Can. J. Genet. Cytol. 23:623-638.

Evans, D.A. and Reed, S.M. 1981. Cytogenetic techniques. *In:* Thorpe, T.A. (ed.). Plant Tissue Culture—Methods and Application in Agriculture. Academic Press.

Forster, B. and Miller, T.E. 1985. A hybrid between diploid *Agropyron junceum* and *Triticum aestivum*. Cereal Res. Commun. 8:355-358.

Friebe, B., Cermeno, M.C., and Zeller, F.J. 1987. C-banding polymorphism and the analysis of nucleolar activity in *Dasypyrum villosum* (L.) Candargy, its added chromosomes to hexaploid wheat and the amphiploid *Triticum dicoccum-D. villosum*. Theor. Appl. Genet. 73:337-342.

Fujigaki, J. and Tsuchiya, T. 1985. Karyotype analysis in a haploid plant of an inbred rye, *Secale cereale* L. by aceto-carmine. Z. Pflanzenzucht. 94:234-243.

Gall, J.G. and Pardue, M.L. 1969. Formation and detection of RNA-DNA hybrid molecules in cytological preparations. Proc. Natl. Acad. Sci. USA 63:378-383.

Geriach, W.L. 1977. N-banded karyotypes of wheat species. Chromosoma 62:49-56.

Gerlach, W.L. and Peacock, W.J. 1980. Chromosomal locations of highly repeated DNA sequences in wheat. Heredity 44:269-276.

Gill, B.S. and Kimber, G. 1974. Giemsa C-banding and the evolution of wheat. Proc. Natl. Acad. Sci. USA 71:4086-4090.

Gill, B.S. and Kimber, G. 1977. Recognition of translocations and alien chromosome transfers in wheat by the Giemsa C-banding technique. Crop Sci. 17:264-266.

Gillam, I.C. 1987. Non-radioactive probes for specific DNA sequences. TIBTEC 5:332-334.

Giraldez, R. and Orellana, J. 1979. Metaphase I Bonds, crossing-over frequency, and genetic length of specific arms of rye. Chromosoma 72:377-385.

Hart, G.E. and Gale, M.D. 1987. Biochemical/Molecular loci of hexaploid wheat (*Triticum aestivum* 2n=42, Genomes AABBDD) *In:* O'Brien, S.J. (ed.). Genetic Maps 1987. A compilation of linkage and restriction maps of genetically studied organisms. Volume 4. Cold Spring Harbor Laboratory.

Helentjaris, T., Slocum, M., Wright, S., Schaefer, A., and Nienhuis, J. 1986. Construction of genetic linkage maps in maize and tomato using restriction fragment length polymorphisms. Theor. Appl. Genet. 72:761-769.

Hsiao, C., Wang, R.R.-C., and Dewey, D.R. 1986. Karyotype analysis and genome relationships of 22 diploid species in the tribe Triticeae. Can. J. Genet. Cytol. 28:109-119.

Hutchinson, J. 1983. *In situ* hybridization mapping of plant chromosomes. In: Brandham, P.E. and Bennett, M.D. (eds.). Kew Chromosome Conference II- Proc. 2nd Chromosome Conference, Kew, England. Sept. 1982. George Allen and Unwin, London.

Hutchinson, J. and Lonsdale, L.M. 1982. The chromosomal distribution of cloned highly repetitive sequences from hexaploid wheat. Heredity 48:371-376.

Hutchinson, J., Chapman, V., and Miller, T.E. 1980. Chromosome pairing at meiosis in hybrids between *Aegilops* and *Secale* species: A study by *in situ* hybridization using cloned DNA. Heredity 45(2):245-254.

Hutchinson, J., Miller, T.E., Jahier, J., and Shepherd, K. 1982. Comparison of the chromosomes of *Triticum timopheevi* with related wheats using the techniques of C-banding and *in situ* hybridization. Theor. Appl. Genet. 64:31-40.

Inagaki, M., Henry, Y., and De Buyser, J. 1987. Comparison of haploid production efficiency through anther culture and intergeneric crossing in three wheat varieties and their F_1 hybrids. Japan J. Breed. 37:474-478.

Islam, A.K.M.R. and Shepherd, K.M. 1981. Production of disomic wheat-barley chromosome addition lines using *Hordeum bulbosum* crosses. Genet. Res. Camb. 37:215-219.

Islam, A.K.M.R., Shepherd, K.W., and Sparrow, D.H.B. 1978. Production and characterization of euplasmic wheat-barley chromosome addition lines. Heredity 46:161-174.

Iordansky, A.B., Zurabishvili, T.B., and Badaev, N.S. 1978a. Linear differentiation of cereal chromosomes. 1. Common wheat and its supposed ancestors. Theor. Appl. Genet. 51:145-152.

Iordansky, A.B., Zurabishvili, T.B., and Badaev, N.S. 1978b. Linear differentiation of cereal chromosomes. 3. Rye, triticale and 'Aurora' variety. Theor. Appl. Genet. 51:281-288.

Jewell, D.C. 1979. Recognition of alien material and chromosome rearrangement in wheat using N-banding. Proc. 5th Int. Wheat Genetics Symp., New Delhi.

Jewell, D. and Mujeeb-Kazi, A. 1982. Unexpected chromosome numbers in backcross I generations of F_1 hybrids between *Triticum aestivum* and related alien genera. Wheat Inf. Serv. 55:5-9.

John, H.A., Birnstiel, M.L., and Jones, K.W. (1969). RNA-DNA hybrids at the cytological level. Nature 223:582-587.

Kimber, G. 1984. Technique selection for the introduction of alien variation in wheat. Z. Pflanzenzucht. 92:15-21.

Kimber, G. and Alonso, L.C. 1981. The analysis of meiosis in hybrids. II Tetraploid hybrids. Can. J. Genet. Cytol. 23:235-254.

Kimber, G. and Hulse, M.M. 1978. The analysis of chromosome pairing in hybrids and the evolution of wheat. Proceedings of the 5th International Wheat Genetics Symposium, New Delhi. pp. 63-72.

Kimber, G., Alonso, L.C., and Sallee, P.J. 1981. The analysis of meiosis in hybrids. I. Aneuploid hybrids. Can. J. Genet. Cytol. 23:209-219.

Knott, D.R. and Dvorak, J. 1976. Alien germplasm as a source of resistance to disease. Z. Pflanzenzucht. 92:15-21.

Koebner, R.M.D. and Shepherd, K.W. 1986. Controlled introgression to wheat of genes from rye chromosome arm 1RS by induction of allosyndesis. 1. Isolation of recombinants. Theor. Appl. Genet. 73:197-208.

Kruse, A. 1969. Intergeneric hybrids between *Triticum aestivum* (V. Koga II, 2n=42) and *Avena sativa* L. (V. Stal, 2n=42) with pseudogamous seed formation. Royal Vet. Agric. Yearbook 1969, Copenhagen Denmark:188-200.

Kruse, A. 1973. *Hordeum* x *Triticum hybrids*. Hereditas 73:157-161.

Landry, B.S., Kesseli, R.V., Farrara, B., and Michelmore, R.W. 1987. A genetic map of lettuce (*Lactuca sativa* L.) with restriction fragment length polymorphism, isozyme, disease resistance and morphological markers. Genetics 116:331-337.

Lapitan, N.L.V., Sears, R.G., and Gill, B.S. 1984. Translocations and other karyotypic structural changes in wheat x rye hybrids regenerated from tissue culture. Theor. Appl. Genet. 68:547-554.

Lapitan, N.L.V., Sears, R.G., Rayburn, A.L., and Gill, B.S. 1986. Wheat rye translocations: detection of chromosome breakpoints by *in situ* hybridization with a biotin-labeled DNA probe. J. Hered. 77:415-419.

Lapitan, N.L.V., Sears, R.G., and Gill, B.S. 1988 Amplification of repeated DNA sequences in wheat x rye hybrids regenerated from tissue culture. Theor. Appl. Genet. 75:381-388.

Laurie, D.A. and Bennett, M.D. 1986. Wheat x maize hybridization. Can. J. Genet. Cytol. 28:313-316.

Lukaszweski, A. and Gustafson, J.P. 1982. Transfer of chromosome arms and segments from rye to wheat. 74th Annual Meetings, American Society of Agronomy. Agron. Abstr. pp. 74.

Lukaszewski, A.J. and Gustafson, J.P. 1983. Translocations and modifications of chromosomes in triticale x wheat hybrids. Theor. Appl. Genet. 64:239-248.

Manuelidis, L., Langer-Safer, P.R., and Ward, D.C. 1982. High-resolution mapping of satellite DNA using biotin-labelled DNA probes. J. Cell Biol. 95:619-625.

Miller, T.E. and Reader, S.M. 1987. A guide to the homoeology of chromosomes within the *Triticeae*. Theor. Appl. Genet. 74:214-217.

Miller, T.E., Hutchinson, J., and Chapman, V. 1982. Investigation of a preferentially transmitted *Aegilops sharonensis* chromosome in wheat. Theor. Appl. Genet. 61:27-33.

Mujeeb-Kazi, A. and Kimber, G. 1985. The production, cytology, and practicality of wide hybrids in the Triticeae. Cereal Res. Commun. 13:111-124.

Mujeeb-Kazi, A. and Miranda, J.L. 1984. High frequency synthetic genome formation potentialities in backcross I selfed derivatives from some intergeneric hybrids involving *Triticum aestivum* and *Agropyron*. 76th Annual Meetings, American Society of Agronomy. Agronomy Abstracts. pp. 80.

Mujeeb-Kazi, A. and Rodriguez, R. 1983a. Cytogenetics of a *Hordeum vulgare* x *Triticum turgidum* hybrid and its backcross progeny with *T. turgidum*. J. Hered. 74:109-113.

Mujeeb-Kazi, A. and Rodriguez, R. 1983b. Meiotic instability in *Hordeum vulgare* x *Triticum aestivum* hybrids. J. Hered. 74:292-296.

Mujeeb-Kazi, A., Roldan, S., and Miranda, J.L. 1984. Intergeneric hybrids of *Triticum aestivum* L. with *Agropyron* and *Elymus species*. Cereal Res. Commun. 12:75-79.

Mujeeb-Kazi, A., Roldan, S., Suh, D.Y., Sitch, L.A., and Farooq, S. 1987. Production and cytogenetics analysis of hybrids between *Triticum aestivum* and some caespitose *Agropyron* species. Genome 29:537-553.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. 15:473-497.

Naranjo, T. and Lacadena, J.R. 1979. Analysis of centromere co-orientation in a rye-wheat derivative by means of C-banding. Chromosoma 73:227-235.

Pardue, M.L. and Gall, J.G. 1970. Chromosome localisation of mouse satellite DNA. Science 168:1356-1358.

Rajaram, S., Mann, CH.E., Ortiz-Ferrara, G., and Mujeeb-Kazi, A. 1983. Adaptation, stability and high yield potential of certain 1B/1R CIMMYT wheats. Proc. 6th International Wheat Genetics Symposium, Kyoto, Japan. pp. 613-621.

Rayburn, A.L. and Gill, B.S. 1985. Use of biotin-labeled probes to map specific DNA sequences on wheat chromosomes. J. Hered. 76:78-81.

Rayburn, A.L. and Gill, B.S. 1986. Molecular identification of the D-genome chromosomes of wheat. J. Hered. 77:253-255.

Rayburn, A.L. and Gill, B.S. 1987a. Use of repeated DNA sequences as cytological markers. Amer. J. Bot. 74:574-580.

Rayburn, A.L. and Gill, B.S. 1987b. Molecular analysis of the D-genome of the Triticeae. Theor. Appl. Genet. 73:385-388.

Sarma, N.P. and Natarajan, A.T. 1973. Identification of heterochromatic regions in the chromosomes of rye. Hereditas 74:233-238.

Schlegel, R. and Gill, B.S. 1984. N-banding analysis of rye chromosomes and the relationship between N-banded and C-banded heterochromatin. Can. J. Genet. Cytol. 26:765-769.

Seal, A.G. 1982. C-banded Wheat Chromosomes in Wheat and Triticale. Theor. Appl. Genet. 63:39-47.

Seal, A.G. and Bennett, M.D. 1982. Preferential C-banding of wheat or rye chromosomes. Theor. Appl. Genet. 63:227-233.

Sharma, H.C. and Gill, B.S. 1983a. Current status of wide hybridization in wheat. Euphytica. 32:17-31.

Sharma, H.C. and Gill, B.S. 1983b. New hybrids between *Agropyron* and wheat. 2. Production, morphology and cytogenetic analysis of F1 hybrids and backcross derivates. Theor. Appl. Genet. 66:111-121.

Sharma, H.C. and Gill, B.S. 1983c. New hybrids between *Agropyron* and wheat. III. Backcross derivates, effect of *Agropyron* cytoplasm, and production of addition lines. Proceedings of the 6th International Wheat Genetics Symposium, Kyoto, Japan. pp. 213-221.

Singh, N.K. and Shepherd, K.W. 1984. Mapping of the genes controlling high-molecular-weight glutelin subunits of rye on the long arm of chromosome 1R. Genet. Res. Camb. 44:117-123.

Sybenga, J. 1983. Rye chromosome nomenclature and homoeology relationships. Z. Pflanzenzuchtg. 90:297-304.

Taira, T. and Larter, E.N. 1978. Factors influencing development of wheat- rye hybrid embryos in vitro. Crop Sci. 18:348-350.

Ter Kuile, N., Asiedu, R. and Mujeeb-Kazi, A. 1987. The role of some cytogenetic systems in effecting alien genetic transfers for *Triticum aestivum* improvement. Agronomy Abstracts pp. 82.

Teoh, S.B. and Hutchinson, J. 1983. Interspecific variation in C-banded chromosomes of diploid *Aegilops* species. Theor. Appl. Genet. 65:31-40.

Teoh, S.B., Hutchinson, J., and Miller, T.E. 1983. A comparison of the chromosomal distribution of cloned repetitive DNA sequences in different *Aegilops* species. Heredity 51:635-641.

Tissue Culture for Crops Project. Progress Report. 1987. pp. 29-33.

Vosa, C.G. 1974. The basic karyotype of rye (*Secale cereale*) analysed with Giemsa and fluorescence methods. Heredity 33:403-408.

International Cooperation on a Major World Crop Genetic Resource: The Latin American Maize Project (LAMP)

Quentin Jones, LAMP Director
Agricultural Research Service
USDA, Beltsville, Maryland;
Ricardo Sevilla, International Research Coordinator for LAMP
Universidad Nacional Agraria
La Molina, Lima, Peru, and,
Wilfredo Salhuana, Senior Advisor to LAMP
Pioneer Hi-Bred, International, Inc.
Plant Breeding Division, Homestead, Florida

Introduction

Maize (*Zea Mays* L.) is native to Central and South America. It is in the countries of these regions that the richest genetic diversity of maize and its closest wild relatives is to be found. At the time Columbus landed at Cuba, all of the more than 200 races of maize known today in the Western Hemisphere were being cultivated by the Amerinds—from Canada to Chile.

The Amerinds, and more recently the colonists from the Eastern Hemisphere, cultivated those races of maize in the areas where the germplasm was best adapted. It is presumed that, like good farmers everywhere who save seed from year to year, they saved the best ears from their garden plots—those "truest-to-type" according to their tastes and experience. As these early maize growers, for whatever reason, moved, from area to area, they took their favorite maize types with them. Coming into contact with locally grown races provided opportunities for natural hybridization and selection of new combinations. Introgression of new genes into the locally adapted races could not have been at the expense of genes or gene complexes conferring adaptation to local environments. But a different range of genotypes interacting with new combinations of environmental factors promoted the emergence of new phenotypes, some of which would attain usefulness and recognition as races.

New phenotypes recognized as having improved traits for productivity or quality would have been favored by farmers and their germplasm preserved through use as a crop in farmers' fields.

The basic rationale or philosophical theme for evaluation of the genetic diversity of maize in LAMP is borrowed from the practices,

LAMP COUNTRIES

1. Argentia
2. Bolivia
3. Brazil
4. Colombia
5. Chile
6. Guatemala
7. Mexico
8. Paraguay
9. Peru
10. Uruguay
11. United States
12. Venezuela

Figure 1.

intuitive or otherwise, of early maize growers. Collectively, they exposed a great diversity of maize germplasm to a wide and changing array of environmental influences and progressively selected the best phenotypes. LAMP, essentially, is following the same course. LAMP is based on the participation and close cooperation of maize scientists from 12 countries (Figure 1). In all of these countries, maize is a very important crop today and has been for perhaps as long as 7,000 years. It has also traveled widely and become a major crop in Central and Southern Europe, large parts of Africa, the Indian subcontinent, and Southeast Asia.

Objectives

The broad objective of the Latin American Maize Project is to systematically evaluate the genetic diversity of maize for genotypes useful in present and future maize improvement programs in all parts of the world. A parallel objective, equally broad, is to develop effective computer-assisted information systems which are able to network internationally. Costly and hard-to-come-by evaluation data is essentially as important as the germplasm upon which it is based. Holders of germplasm and users of germplasm must be able to communicate freely and accurately, or otherwise neither the germplasm nor the information will be used.

To meet these broad objectives, a plan of work has been developed and agreed upon which details the protocols and procedures for selecting maize accessions for entry into the project, selection of experimental sites at which different accessions should be planted to meet their ecological requirements, planting plans, including numbers of replications, permissible agronomic practices, time and method for scoring each descriptor, and managing and reporting the derived data.

Each country decides which of its qualified accessions it will enter into LAMP; designates the Principal Investigator (PI), who will be responsible for LAMP work in that country; and designates an Information Specialist (IS), who works closely with the PI and is the person responsible for data management. Table 1 provides the names of participating institutions, Principal Investigators, and Information Specialists. Table 2 presents the evaluation data sheet used by all LAMP participants. Table 3 provides definitions of descriptors and descriptor states.

All Principal Investigators in LAMP, as well as all Directors of cooperating institutions, have indicated a keen desire to accomplish LAMP objectives and to communicate the resulting scientific data as widely and as completely as possible.

Table 1. Participating institutions in LAMP.

Country	Institution	Principal Investigator	Information Specialist
Argentina	Instituto Nacional Tecnologico Agropecuario	Nestor Baracco	Edit Frutos
Bolivia	Centro Investigaciones Fito-ecogeneticos	Gonzalo Avila	Mario Crespo
Brazil	Centro Nacional de Recursos Geneticos	Jairo Silva	Eduardo V. Morales
Colombia	Instituto Colombiano Agropecuario	Fernando Arboleda	Orlando Martinez
Chile	Instituto Investigaciones Agropecuarias	Orlando Paratori	Fernando Silva
Guatemala	Instituto de Ciencia y Technologia Agricolas	Carlos Perez	Juan Manuel Herrera
Mexico	Instituto Nacional Investigaciones Forestales, Agricolas y Pecuarias	Francisco Cardenas R.	Francisco Cienfuegos
Paraguay	Dir. Investigacion y Exper. Agropecuarias y Forestal	Mercedes Alvarez	Luiz Alvarez
Peru	Prog. Cooperativo de Invest. en Maiz; Univ. Nac. Agraria	Ricardo Sevilla	Walter Fegan
Uruguay	Facultad de Agronomia	Grisel Fernandez	Monica Cadenazzi
U.S.A.	Corn Breeding USDA/ARS Iowa State Univ., Ames	Linda Pollak	Linda Pollak
Venezuela	Fondo Nacional de Invest. Agropecuarias	Arnoldo Bejarano	Victor Segovia

Table 2. Evaluation Data Sheet Used by All LAMP Participants.

													PARCELA PLOT
													ENTRADA ENTRY
													PEDIGREE
													NO. DE PLANTULAS NO. OF SEEDLINGS
													DIAS A LA FLORACION MASCULINA NO. OF DAYS TO TASSEL
													DIAS A LA FLORACION FEMENINA NO. OF DAYS TO SILK
													ALTURA DE LA PLANTA PLANT HEIGHT
													ALTURA DE LA MAZORCA EAR HEIGHT
													NO. DE PLANTAS NO. OF PLANTS
													NO. DE PLANTAS QUEBRADAS NO. OF BROKEN STALKS
													NO. DE PLANTAS TUMBADAS NO. OF ROOT-LODGED PLANTS
													GRADO DE MACOLLAMIENTO DEGREE OF TILLERING
													NO. DE MAZORCAS NO. OF EARS
													CALIDAD DE LAS MAZORCAS EAR QUALITY
													PESO EN EL CAMPO FIELD WEIGHT
													PORCENTAJE DE HUMEDAD MOISTURE PERCENT
													TIPO DE GRANO KERNEL TYPE
													COLOR DEL GRANO KERNEL COLOR
													NOMBRE DE LA RAZA RACE NAME

Table 3. Definitions of descriptors and descriptor states.

Number of seedlings: count of plants prior to thinning.

Number of days to tassel: record days from planting to 50 percent shed.

Number of days to silk: record days from planting to 50 percent silk.

Plant height: height of the plant in centimeters. This is based on average measurements for 10 plants from ground level to top of tassel.

Ear height: this measurement is taken at the same time as plant height. This measurement is taken from ground level to the vertex of the angle of insertion of the uppermost developed ear on the stalk.

Number of plants: count the number of plants in each plot.

Number of broken stalks: count of stalks in each plot that have broken below the ear before corn is picked.

Number of root-lodged plants: count of plants leaning 30 degrees or more in the first 60 cm. from the ground.

Degree of tillering. code indicate the amount of tillers. 1.-none; 9.-high.

Number of ears: count the number of ears in each plot.

Ear quality: code indicating the soundness. 1.-very poor; 9.-excellent.

Field weight: read scales to the nearest tenth of a kilogram.

Moisture percent: moisture samples may then be taken by shelling two rows from each of 10 ears.

Kernel type: code indicating the type of kernel.

FR	= floury	SF	= semiflint
DE	= dent	PP	= pop
SD	= semident	SW	= sweet
FT	= flint		

Kernel color: coding indicating the color of kernel.

AM	= yellow	RM	= red mosaic
BL	= white	MA	= brown
NA	= orange	MC	= brown mosaic
RO	= red	MV	= brown variegated
RV	= red variegated	PR	= purple

Race name: names of the races pertaining to the collections.

Plan of Work

The success of LAMP will be largely dependent upon how well the participating scientists in each country follow agreed plans and protocols. The United States and the eleven Latin American countries will each have several scientists participating in LAMP, as well as a number of field and laboratory technicians. Additionally, each country will be conducting evaluation work at several sites that have different environmental parameters. In total, LAMP involves 30-40 scientists working at 35-40 sites distributed over 70° of latitude and ranging from sea level to over 3,300 m (Figure 2).

The germplasm being evaluated in LAMP also represents enormous diversity. Most, if not all, of the recognized races of maize are included in the study. In Stage I, 15,546 accessions were subjected to preliminary evaluation.

The only factors involved in this project that can be held constant across locations and accessions are those governing experimental design, protocols of data taking and analysis, and information. Therefore, the Cooperative Agreement and Plan of Work were designed to clearly define these factors and communicate them to participating scientists and administrators. The Principal Investigators of LAMP, based on experience gained in Stage I, have agreed on some rather minor but important changes in plans and protocols involving exchanges of accessions of germplasm among countries prior to Stage III and on into Stages IV and V.

Evaluation Procedures

Information provided here serves the purpose of describing the rationale for evaluating a large number of accessions of a given crop for a rather extensive number of descriptor categories, over a wide range of environmental parameters, involving the cooperation of more than a few countries, while attempting to achieve reliable results on a modest budget. Of course, it is hoped that LAMP will be highly successful in achieving its goals and that concerned scientists dealing with other crops will be encouraged to undertake needed germplasm evaluation programs.

Stage 1 [1]

Each accession selected in a country is planted in two replications, 10-m. row each, at a single location that best matches the environ-

[1] A "stage" involves one cropping season. Preparation for planting, postharvest processing of data, and preparation of seed and detailed planting schedules for the following stage require approximately one calendar year.

COUNTRY	POLITICAL DIVISION		LOCATION
1 BOLIVIA	Cochabamba	1.1	Pairumani
2 BRAZIL	Minas Gerais	2.1	Sete Lagoas
		2.2	Janauba
		2.3	Pelotas
3 COLOMBIA		3.1	Turipana
		3.2	Palmira
	Antioquia	3.3	Tulio Ospina
	Antioquia	3.4	La Selva
		3.5	Tibaitata
4 CHILE	Santiago	4.1	La Platina
		4.2	Copiapo
5 GUATEMALA		5.1	Cuyuta
		5.2	Chimaltenango
		5.3	Quezaltenango
6 MEXICO	Mexico	6.1	Chapingo
		6.2	Celaya
	Veracruz	6.3	Cotlaxtla

COUNTRY	POLITICAL DIVISION		LOCATION
7 PARAGUAY	Cordillera	7.1	Caacupe
	Itapua	7.2	Capitan Miranda
8 PERU	Piura	8.1	Piura
	Lima	8.2	La Molina
	Ancash	8.3	Caraz
	Ancash	8.4	Carhuaz
	Cuzco	8.5	Calca
	Junin	8.6	Jauja
	Huanuco	8.7	Huanuco
9 URUGUAY	Paisandu	9.1	Paisandu
10 VENEZUELA	Maracay	10.1	Maracay
11 U.S.A.	Iowa	11.1	Ames
	Georgia	11.2	Tifton
	Puerto Rico	11.3	Isabela

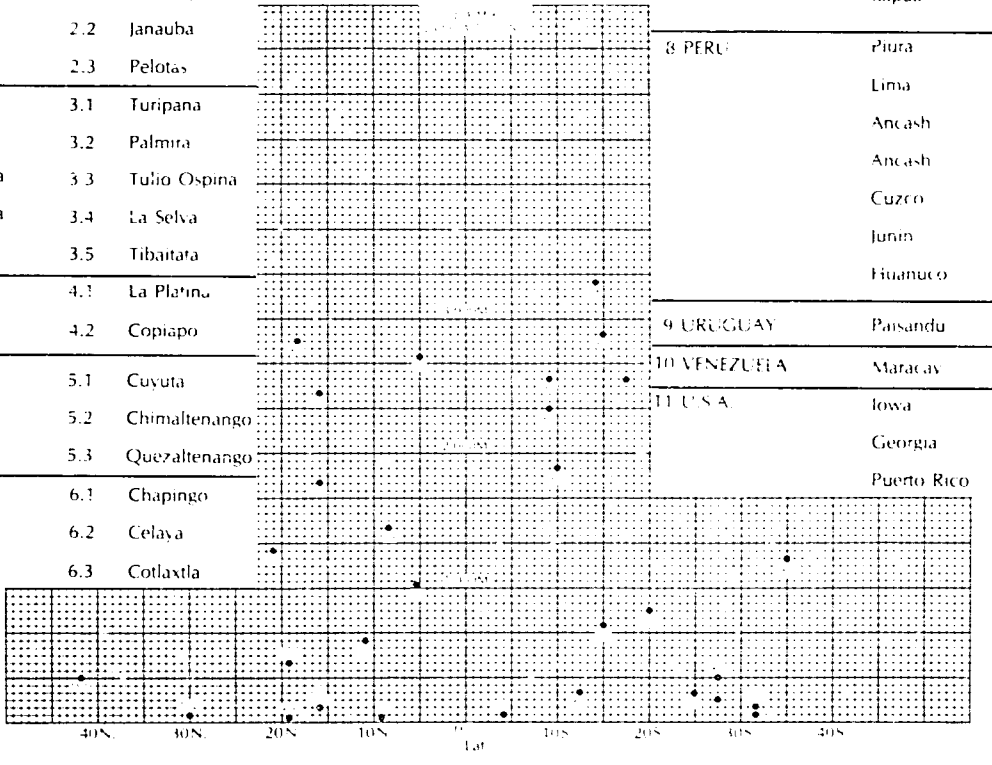


Figure 2. LAMP Locations grouped by altitude.

ment in which the accession was originally collected. Accessions lacking original location data are planted in all altitudinal regions (one 10-m. row at each site) in that participating country to determine the environment that best suits their individual requirements.

If plant height and maturity are known, accessions are grouped to avoid interaccession competition and to facilitate data acquisition. Of course, if races to which accessions belong are known, desirable grouping can be made on that basis. One or two locally adapted checks are randomly distributed as rows among every 20 to 30 rows of accessions.

Characteristics evaluated in this stage are:

- number of seedlings
- male and female flowering data
- plant and ear height
- number of mature plants
- number of plants root-lodged, stalk-lodged, and degree of tillering
- number of ears, field weight, moisture content, ear quality
- kernel type, color
- race

In addition to these descriptors, the evaluator can add others that he considers desirable.

The field data book is organized to facilitate error-free transfer of the collected data to the computer for later analysis, exchange, and publication.

On the basis of results from this stage, 20 percent of the accessions are selected, per altitudinal region, for further evaluation in Stage 2.

Stage 2

The upper quintile (top 20 percent) of accessions evaluated in Stage 1 will be planted and grouped for maturity in yield tests at two locations per altitudinal region with two replications per location, including checks. The characteristics to be evaluated in this stage are the same as for Stage 1. At the same time, evaluation of special factors, (e.g. disease resistance, insect resistance, aluminum toxicity factors, etc.), will be accomplished. However, some of these special factor evaluations will have to be done in fields well separated from those where regular evaluation studies are being undertaken.

In these special evaluation plantings, maturity groups are planted together at one location, one row per accession. At desired inter-

vals, checks known to be susceptible, intermediate, or tolerant are included in order to establish the special factor incidence. Tolerant and intermediate accessions will be selected for further evaluation in other sites and countries.

On the basis of results from Stage 2, 5 to 10 percent of accessions will be selected for continuing evaluation in Stage 3.

Stage 3

This stage utilizes the top 5 or 10 percent of accessions selected in each country on analysis of results from Stage 2. These selected accessions are interchanged among countries having similar environmental regimens (homologous areas) (Table 4). Each country will evaluate these exchanged selections at two locations with two replications per location. An additional procedure will be to cross these selections in an isolated field with a tester that is adapted to that locality. The selected accession will be the female parent and will be detasseled at the moment of flowering. The adapted tester will be the male in the cross.

The selection of the tester is very important; hence, several reasons for making these crosses must be considered:

- Yields of the accessions indicate the degree to which they possess genes favoring yield, but evaluation of crosses that involve accessions and a known tester would indicate the degree to which accessions contribute favorable genes different from those known to be in the tester varieties.

- Nonadditive gene action may be important.

- The tester may give adaptability to the foreign accession and thus permit more realistic comparisons with native accessions.

- Crosses may reveal new heterotic patterns.

- Using the accession as the female in the cross permits the opportunity of picking up valuable cytoplasmic genes.

Also in Stage 3, special factor evaluation for insect or disease resistance or soil reactions will be continued. All the intermediate and resistant accessions will be planted again in appropriate locations in countries having an interest in them. Figure 3 displays the maize calendar for LAMP locations.

Table 4. Seed interchange among homologous areas (Stage 3 of LAMP).

CRITERIA FOR SEED INTERCHANGE:

FIVE HOMOLOGOUS REGIONS HAVE BEEN DEFINED, COMBINING TWO CHARACTERISTICS OF LOCATIONS WERE STAGE 3 EVALUATION WILL BE DONE: *ALTITUDE* AND *LATITUDE*, WITH THE FOLLOWING PARAMETERS:

ALTITUDE:
1. 0 TO 1,200 M.
2. 1,200 TO 1,800 M.
3. 1,800 TO 2,400 M.
4. HIGHER THAN 2,400 M.

LATITUDE:
A. SHORT DAYS, 0 TO 23 DEGREES
B. LONG DAYS, GREATER THAN 23 DEGREES

USING THESE CRITERIA, THE FOLLOWING HOMOLOGOUS AREAS WERE ESTABLISHED AND LOCATIONS ASSIGNED FOR INTERCHANGE:

1A. SETE LAGOAS — JANAUBA — TURIPANA — PALMIRA — CUYUTA — COTAXTLA — PIURA — CAACUPE — LA MOLINA — MARACAY — PUERTO RICO — SANTA CRUZ — HUANUCO — CAPITAN MIRANDA

2A. TULIO OSPINA — CHIMALTENANGO — CELAYA — LA MOLINA — HUANUCO — CARAZ

3A. PAIRUMANI — TULIO OSPINA — LA SELVA — CHIMALTENANGO — QUETZALTENANGO — CHAPINGO — CARHUAZ

4A. LA SELVA — TIBAITATA — CUZCO — JAUJA — PAIRUMANI

1B. PELOTAS — LA PLATINA — COPIAPO — CAPITAN MIRANDA — PAYSANDU — IOWA — GEORGIA

Stage 4

Each country will plant an experiment utilizing 1) the crosses of the accessions with all testers, 2) the accessions, 3) the testers, and 4) the checks. The experiment will be planted in each country at three locations with two replications per location. The results of the analyses will provide good leads to those accessions having promise for future breeding programs.

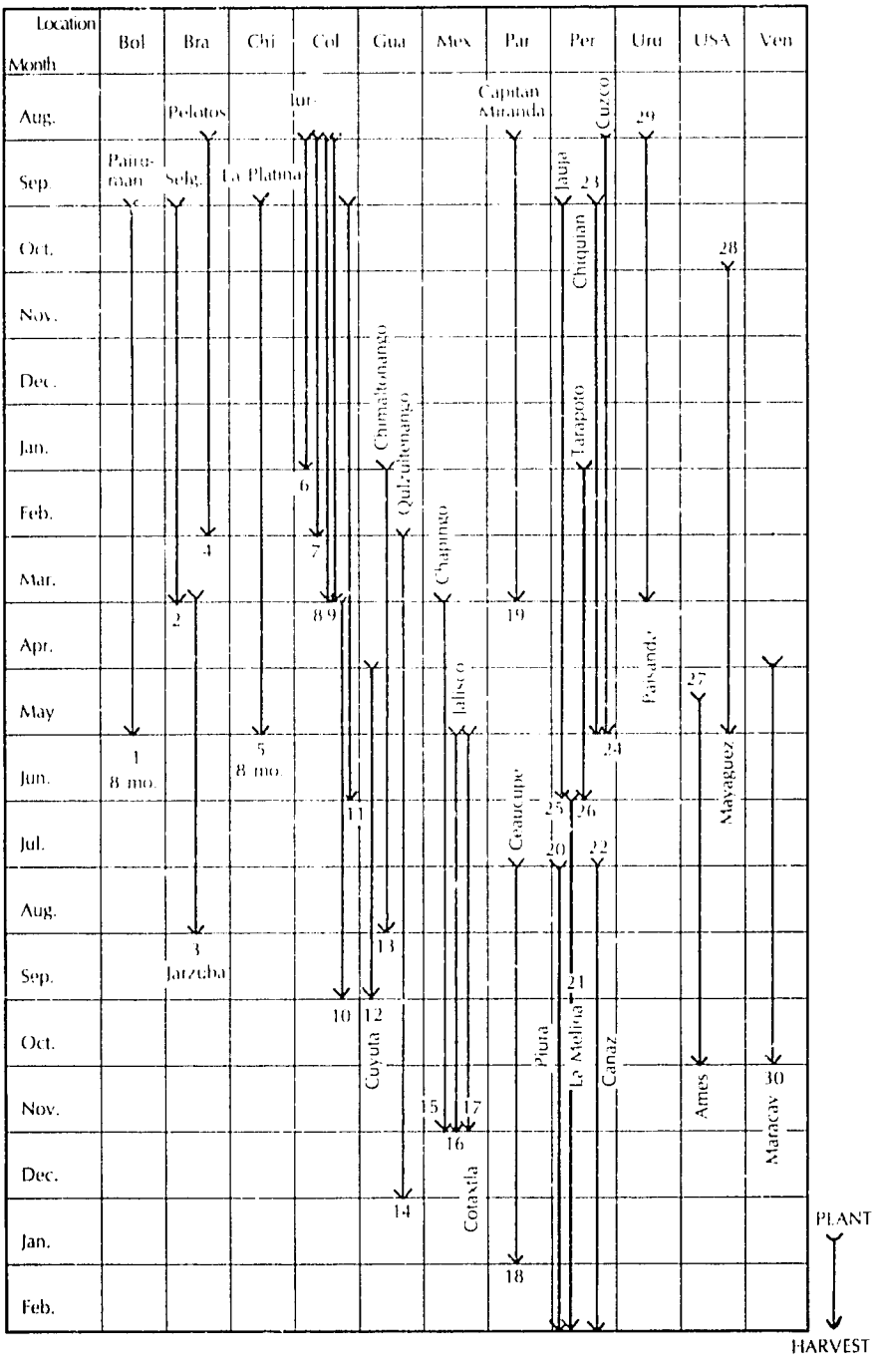


Figure 3. Maize growing season cycles at LAMP locations.

Stage 5

Breeders in each country will use enhanced germplasm from Stage 4 that shows high potential for helping them reach their breeding program objectives.

Data Capture, Analysis, and Exchange

The importance of data acquisition, analysis, use, and exchange cannot be overemphasized. Information Specialists in LAMP need to fully understand LAMP objectives and be actively involved in the full range of activities described in the Plan of Work.

An objective in the overall coordination of LAMP is to insure a working level of intercompatibility of information systems among all LAMP countries. This objective is being met through on-the-job training and through upgrading of information equipment as necessary.

Reporting

An annual technical report is required consisting of four parts:

1. A complete set of evaluation data sheets for each stage.
2. Data analyses leading to selection of accessions to be further evaluated in later stages.
3. Citation of accession entries displaying unusual performance or traits.
4. Identification of problem areas that may affect subsequent work.

Summary Observations

Stage 1 of LAMP has been completed; most of the countries are well into Stage 2, and detailed planning for Stage 3 (allowing for the exchange of accessions among countries for further evaluation) is in progress. Much has been learned beyond the program's objective of evaluating maize germplasm. For example, when those countries maintaining maize gene banks examined the status of their holdings in some detail for the purpose of determining availability of the accessions for entry into LAMP, it was found that many accessions were represented by dead seed or seed of very low viability or too few seed to meet even Stage 1 requirements. Some of these accessions can be traced back to collections made in the 1940s and 1950s whereas some are of more recent acquisition. There are two principal reasons for this unfortunate situation. One is inadequate budgets for germplasm maintenance, and the other (which may in part be due to inadequate budgets) is that gene bank

managers too often lack necessary training and skills. Whatever the reason or reasons, irreplaceable genetic resources are being lost.

International agricultural development agencies and commercial agricultural enterprises should be made aware of the critical need to assist developing countries with the costs of maintaining active collections of germplasm of their principal crops. The modest costs of training of germplasm maintenance personnel, plant breeders, and agronomists must be met from outside most developing countries.

Sustainable agricultural production systems require soil, water, sunlight, and adapted crop germplasm, for use by trained agricultural scientists. These necessities, plus supportive government policies, can make the Green Revolution a reality in most regions of the world. Farmers who do not have access to seed of commercial varieties adapted to their production environments save seed from their own crops for planting the following year. While this is a good way to maintain germplasm of adapted local races or subraces, the farmer will usually not be getting the return from his investment that he could from varieties developed for his production area by skilled maize breeders.

Governments of developing countries need to be encouraged to place much higher priority on crop production systems. Perhaps, agricultural development aid—for irrigation projects, soil improvement, and infrastructural needs (transportation, supplies, marketing, credit, etc.)—could be made contingent upon continuing progress in crop germplasm maintenance, improvement, and use under state-of-the-art agronomic practices.

Sustainable agriculture must have these ingredients. Biotechnology will be another important addition to support the technologies of modern agriculture, but it, too, requires good genes, good science, and good farmers.

Legal Protection of Plant Varieties

Stanley D. Schlosser
Attorney At Law
Alexandria, Virginia

The Economics of Commercial Plant Breeding

Throughout history, plant breeding depended primarily on a combination of folk knowledge, personal observation, available scientific information and plant materials from previous harvests. While desirable new varieties sometimes resulted from traditional breeding of available plant germplasm, farmers were often reluctant to risk adoption of new varieties.

The unpredictable nature of plant breeding thus kept it from becoming a major business activity until a more scientific approach was established in this century. Today, plant breeding is a science-driven, research-oriented industry, involving substantial payrolls, extensive laboratory and field programs and sizeable operating expenses. This financial and scientific commitment constantly results in development and marketing of new and better plant varieties.

Nevertheless, the risks and uncertainties of plant development may rival those of the pharmaceutical industry, and prices for agricultural products must include the costs of both successful and unsuccessful projects in order to return a reasonable profit.

Future R&D investments in plant breeding by investors can be encouraged if there are legal mechanisms to keep new varieties out of the hands of copiers. It is well known that copiers, having no research or development costs, and often free of advertising expenses as well, can underprice seed companies supporting their own breeding programs and gain market dominance. When meaningful legal protection becomes available and competition replaces piracy, private investments in research and development increase dramatically. Public and private nonprofit university and foundation research will, of course, continue. But even these sources of new technology are directing their efforts more and more to commercial products. They can afford to promote scientifically-oriented research without the need or inclination for profit generation.

It is also risky to rely too heavily on government sponsorship of scientific research. Government agencies may select research projects on the basis of political, fiscal, and other non-scientific con-

siderations. While one group may lobby for research to eradicate a plant virus threatening the nation's grain production, the government may choose to favor the petition of another group that wants available research funds to improve exportable crops such as coffee or tobacco. This is not an argument for excluding governments from participation in crop improvement and biological research. Government agencies should be encouraged to continue and expand their traditional roles. Governments should not, however, carry the entire research burden. There is room for an important private sector role.

The International Legal System

An established, but far from uniform, international legal system for protecting new plant varieties and plant developments already exists. Participants in that system include the United States, most of Western Europe, New Zealand, Japan, and Hungary. Australia and Poland are expected to joint these ranks. Developing countries have for various reasons been reluctant to participate.

Plant breeders' rights systems are in effect in most of Western Europe. Patents are the other major system for protecting plants, but their protective system must sometimes be adjusted to meet the unique requirements of biotechnology. These systems have some features in common as well as embody important differences. Both systems provide the breeder with an exclusive marketing term (usually about 15 to 20 years). To obtain protection for a variety, it must be proven to be distinct, stable, and uniform. This is usually done through a government testing program, without the breeder's participation. Patent systems for protecting new plant varieties, such as those of Italy and Hungary, impose the same general criteria. The United States patent system, however, applies to plant varieties the same criteria as for other inventions—they must be new, useful, and unobvious.

The United States, in fact, provides a choice of systems for protecting new plants. Asexually propagated varieties are protectable under a special plant patent law. Sexually reproduced varieties, except F1 hybrids, are protectable under the Plant Variety Protection Act, administered by the Department of Agriculture. Any plant variety, plant genus, or species, may be patented under the utility patent law, however, even if also protectable under another law.

The Plant Variety Protection Act is essentially a breeders' rights law, although it resembles the patent law in form and organization and offers protection for plant varieties shown to be distinctive, uniform

and stable. These characteristics are established on the basis of tests conducted by the breeder himself, thus saving the costs and time required for government testing.

Three multilateral agreements regulate the international system. The Paris Convention for the Protection of Industrial Property is the oldest and most general. This Convention, assuring the rights of national treatment for a foreign national of a member state seeking or enforcing rights in other member states, is adhered to by almost one hundred countries. It is concerned with intellectual property generally, and not directed particularly to biotechnology.

The Budapest Treaty concerns the deposit of samples of micro-organisms in connection with patent applications. One requirement for obtaining a patent is inclusion in the patent specification of an "enabling disclosure." This disclosure must be complete enough to enable a person of ordinary skill in the involved technology, using no more than reasonable experimentation, to make and use the invention. For mechanical, electrical and chemical inventions, a written description, accompanied by drawings, is adequate to meet this enablement requirement. Living matter, however, cannot usually be described by words alone, as even the simplest organism is far more complex than the most complicated machine. Reproduction and use of a biotechnological invention, therefore, usually require the availability to researchers of a sample of the biological material used in the invention.

This Treaty establishes a system of internationally recognized depositories for patent purposes called International Depositary Authorities, where deposits of such biological materials may be made and from which samples will be available to the public upon the grant of a patent. Many national patent systems, of course, recognize depositories other than International Depositary Authorities.

The most important international agreement on the protection of plant varieties is the Convention of the International Union for the Protection of New Varieties of Plants. This UPOV Convention is now being revised, the last previous revision having occurred in 1978. This 17-nation Convention requires its member states to provide a minimum standard of protection for varieties, and states the criteria and standards on which protection depends.

The UPOV Convention authorizes protection either by means of patents (meeting the requirements and criteria of the Convention) or breeders' rights. It prohibits, however, the protection of a single

species by both systems. Breeders, for the most part, believe they should have a choice of systems, depending on their particular circumstances and needs. This question of dual protection awaits resolution.

The scope of protection required by the Convention is generally regarded as inadequate. A remedy is anxiously sought by the member states who are authorized, and even encouraged, to raise the minimum standard, even though few have done so. An expected result of the Convention revision is the setting of a higher mandatory standard of protection.

The UPOV minimum standard requires protection only against the unauthorized sale of propagative material as such. Sale of propagative material for other purposes, e.g., the sale of wheat for milling, is not an infringement. Nor is protection required against the unauthorized reproduction or sale of parts or products of protected plants. Most likely, the new Convention will provide protection against any unauthorized use or sale of a protected plant.

Unauthorized commercial reproduction is cheap, efficient and easy. Not even hybridized varieties remain immune from the need for legal protection. It may once have been true that hybrids had their own automatic legal protection. As long as the inbred lines could be kept away from competitors, the variety could not be reproduced. Today, however, techniques, such as micropropagation, enable hybrids to be reproduced without using the lines from which they were originally bred.

Certain exceptions to infringement will probably be retained. Both the European members and the United States include in their laws a farmer's privilege. This privilege, in the European version, authorizes a farmer to multiply seed for his own later use without incurring liability. In the United States, the privilege goes a step further. Multiplied seed can even be sold to a neighboring farmer.

There is almost a certainty of retaining in one form or another the present right of a researcher to experiment with a protected variety in order to develop a new variety. Repeated use of the protected variety to produce the new variety in commercial quantities, however, would continue to be an infringement.

UPOV officials have debated for many years, but still not resolved, the best way of distinguishing one variety from another. In fairness to the member states, however, this is an exceedingly complicated and argumentative issue.

The debate centers on the characteristics to be examined for each species. It is agreed that different characteristics should be examined for different species, but that is where agreement more or less ends. No matter which characteristics are selected, it is recognized that other intentionally disregarded characteristics could have been used.

The question of quantifying the amount of difference or distinctness needed to constitute a new variety remains. Should the difference be small or large? Should a number of lesser distinctions count for the same, more, or less, than a major distinction in one characteristic? Should agronomic features count for more than other features? These issues are collectively referred to in UPOV circles as the "minimum distance" problem.

A small minimum distance requirement makes it easy to obtain protection, but protection is hard to enforce. Slight differences between the protected variety and the variety accused of infringement can almost always be found.

On the basis of these slight differences, one can argue that the accused variety is not the infringing variety, but a distinct new one. A large minimum distance requirement creates exactly the opposite situation. It becomes harder to obtain protection, but protection, once obtained, is easier to enforce.

Perhaps the most difficult task facing the member states is resolution of the overlap between industrial patents and breeders' rights. Industrial patents cover inventions such as plant genera and species, plant breeding methods (under most patent laws these cover a plant bred by a patented method), and even genetic sequences contained in a new plant variety. Breeders' rights apply only to new varieties, but a new variety may contain a patented gene sequence or belong to a patented genus or species, or be produced by a patented process. Hence, the overlap.

There are polarized positions on this question. Advocates of breeders' rights, who largely rely on traditional crossbreeding for the development of new varieties, believe, in general, that their crossbred varieties should have the unhampered benefit of plant breeders' rights. The genetic engineers, who are far more likely to develop broader inventions, argue that they are entitled to traditional patent rights.

There is a common misconception that breeders' rights and plant patents cause the disappearance of varieties and erosion of the genetic base on which research depends. Actually, the introduction

of new and better varieties has the beneficial affect of displacing inferior varieties from commercial production and distribution channels.

Nevertheless, there is great, if not immeasurable, value in preserving these older, displaced varieties. Their preservation in gene banks is completely consistent with either breeders' rights or a patent system and should be vigorously carried on by foundations and governments.

Sound public policy dictates, however, that these collections should include only publicly available materials. There must not be any requirement for the involuntary inclusion of proprietary breeding material. Otherwise, hard-earned and valuable private property will be given away to competitors. The best insurance against genetic erosion is a strong plant breeding industry, which depends on the retention of proprietary varieties by their owners.

A New Strategy

Economically efficient plant breeding, like any scientific research, benefits from the sharing of new technology. Isolated research and reinvention waste money, time, and personnel.

There are many ways to acquire and share new technology, but one of the best is to provide legal protection for plants. Model laws for doing this are even available from the UPOV Secretariat.

The cost of licensing or buying new technology is usually less than the cost of independent development. Major technology developers are reluctant, however, to export technology to countries where it is not protected. They will also be inclined to develop technology for regions or specific geographic conditions for which protection is available.

Despite developing country concerns, foreign companies do not obtain patents or breeders' rights solely or mainly to prevent independent local development and use of the protected technology. Under most of these protection systems, an owner of legal rights cannot sit on them to suppress the introduction of new technology. The owner must exercise his rights within a reasonable time or see them forfeited for "non-working."

There is no compelling business reason why breeders and breeding centers should not utilize the international legal system. Only tradition and, perhaps, a reluctance to receive royalties from a country

that contributes to a center's support, dictate otherwise. Legal protection, however, need not be the exclusive instrument of foreign businesses. When a breeding center owns legal rights, there will be enhanced opportunities for exchanging technology.

Profit generation from the exercise of legal rights can be an important asset. Although profits should not become the main impetus to research, they can be used to fund further research endeavors.

Joint ventures and other business arrangements between international breeding centers and developed country ag-businesses, universities and research institutions should be evaluated to determine where the ownership of legal rights may be needed. These sorts of arrangements offer opportunities to capitalize on the expertise of local scientists and other professionals. At the same time, otherwise unaffordable research can be conducted.

Plant Genetic Resources Network in India

Rajendra Singh Paroda
Deputy Director General (Crop Sciences)
Indian Council of Agricultural Research
New Delhi 110001 (India)

Plant genetic resources are the foundation of any crop improvement process. They need to be conserved in the interest of human welfare. Concern for the collection, evaluation, utilization and conservation of these resources has been expressed in the recent past throughout the world. Man's intervention with nature is resulting into elimination of several useful plant species/forms. Thus, conservation of these resources is of paramount importance.

Indian Gene Centre

The Indian Gene Centre holds important position in the international network of plant genetic resources mainly on account of two factors. Firstly, India is one of the eight primary centres of origin/crop diversity as recognised by Vavilov (1949-50). Secondly, India took an early initiative to embark upon plant introduction and collection activities. Moreover, the Indian subcontinent is also considered to be an important cradle of agriculture.

Zeven and de Wet (1982) considered that nearly 2,400 plant species of economic importance could be accounted in 12 mega gene centres/regions of diversity. Of this, 160 species are under cultivation in India including both indigenous and naturalized exotics. Besides, well over 320 wild related species have been reported in India (Arora and Nayar, 1984). However, in the global context, where about 600 major and minor food and fodder crop plants are presently being utilized, only about 30 species are considered to be most important (Harlan, 1975).

The genetic resources of the Indian Gene Centre are distributed in eight phyto-geographical/agro-ecological zones and these exhibit considerable preponderance of variable forms/land-races/primitive types in different crops in cereals, millets, legumes, vegetables, fruits, oil seeds, fibres, sugar yielding types, spices, condiments and others. The important crops in which rich land race diversity exists include *Oryza sativa* var. *indica* and several wild *Oryza* species, *Saccharum officinarum* and allied genera, *Citrus* spp., *Musa* spp. *Magnifera indica* and other species, jack fruit, *Solanums*, cucumber, ridge gourd, bitter gourd, okra, oil seed *Brassicae*, sesame, saf-

flower, *mung*, *urad*, rice bean, moth bean, pigeon pea, cowpea, jute, tree cotton, several spices and condiments, taros and yams.

National Bureau of Plant Genetic Resources

The Division of Plant Introduction, Indian Agricultural Research Institute, New Delhi, played an important role and made highly valuable contributions in the sixties through the introduction and collection of plant genetic resources. It was mainly after the creation of the National Bureau of Plant Genetic Resources (NBPGR) in 1976 that activities relating to plant genetic resources received required impetus.

The National Bureau of Plant Genetic Resources is located on the campus of the Indian Agricultural Research Institute (IARI), under the aegies of the Indian Council of Agricultural Research (ICAR). At its headquarters NBPGR is organised through five Divisions; (1) Germplasm Exchange, (2) Plant Quarantine, (3) Plant Exploration, (4) Germplasm Evaluation, (5) Germplasm Conservation. Besides, a special project entitled the National Facility for Plant Tissue Culture Repository was established in 1986 with the financial support by the Department of Biotechnology. At its headquarters, the Bureau has main administrative offices, laboratories, long-term conservation facilities as well as the Quarantine Isolation Nursery. An experimental farm of 40 hectares is located at Issapur, about 40 km. away from the headquarters. In addition, NBPGR has five regional stations in different agroclimatic zones, each being headed by a senior scientist (botanist). These are Shimla (temperate northwestern Himalayan belt), Jodhpur (arid zone), Akola (semiarid region), Trichur (humid tropical zone) and Shillong (northeastern subtemperate/subtropical humid region). Ten plant exploration base centres have also been created at each of these regional stations and also at New Delhi, Bhowali, Ranchi, Cuttack and Hyderabad. Two more exploration centres are being established, one at Srinagar (Jammu & Kashmir) for the temperate and alpine region and another at Port Blair in the Andaman and Nicobar Islands. NBPGR presently operates with a total strength of 542 staff (scientific 151, technical 108 and others 283).

Three All India-Coordinated Projects are also located at NBPGR headquarters. These include: (1) All India-Coordinated Project on Medicinal and Aromatic Plants, (2) All India-Coordinated Project on Under Utilized and Under Exploited Plants and (3) All India-Coordinated Project on *Guar* (Cluster bean).

The main objectives of NBPGR are: (a) Introduction and exchange of germplasm from national/international organisations, under strict phytosanitary conditions and quarantine control to salvage the infected/infested materials, (b) To undertake and coordinate national and international explorations, evaluate/characterise and document the germplasm and to coordinate with crop institutes, coordinated projects, agricultural universities, international institutes, (c) To conserve germplasm collections for medium-term and long-term storage (base collection) in the National Gene Bank as well as in the National Plant Tissue Culture Repository, (d) To impart training in the field of plant resources and associated disciplines.

Actual Achievements

(i) Plant Exploration and Germplasm Collection

NBPGR executed effectively several region-specific multicrop collections and crop-specific missions and potential genetic variability has been captured. Well over 50,000 germplasm accessions have been assembled in agrihorticultural crops (Paroda and Arora, 1986).

During the past one decade (1976-87), the NBPGR successfully organised joint foreign expeditions in India. The important among these include the Indo-Japanese expedition for minor millet germplasm (1985 and 1987), the Indo-Australian Expedition (1984) for collection of wild forage and legumes from black, heavy clay and acidic soils in central and peninsular India, and the NBPGR-IRRI joint expedition in the Western Ghats, India for collection of wild *Oryza* species.

(ii) Introduction and Exchange

Introduction and exchange of germplasm has received considerable impetus on account of established linkages with over 80 countries as well as several CGIAR-supported international institutes like IRRI (Philippines), CIMMYT (Mexico), ICARDA (Syria), CIP (Peru), CIAT (Colombia), ICRISAT (India), IBPGR (Italy) etc. Besides, germplasm exchanges have taken place between India and USDA (USA), CSIRO (Australia), AVRDC (Taiwan), INTSOY (USA), VIR (USSR), INRA (France) and several others. The exchange of genetic resources during 1976-86 included 81,472 introductions/accessions.

(iii) Quarantine Activities

NBPGR plays a significant role as far as quarantine activities for research materials are concerned since the Plant Protection Adviser, Government of India has delegated this authority to NBPGR. The

Division of Plant Quarantine, after interception, tries to salvage the valuable germplasm. NBPGR also provides quarantine services to ICRISAT for germplasm exchanges at the global level. It also deals with the trial materials received from CGIAR Centres such as IRRI, CIMMYT, CIP, CIAT, ICARDA, etc.

The exchange of seeds/plant propagules on a global basis has resulted in the movement of pests/pathogen from their native habitats to the newer areas/locations. The Bureau has played a very effective role in plant quarantine service and during 1976-86, a wide variety of agrihorticultural crops (53,900 samples) were introduced into India. Several important exotic plant pests, nematodes, and pathogens were also intercepted. Some of the important ones include: Insects—*Acanthosceiides obtectus* (bruchid on cowpea and pigeon pea), *Specularius erythraeus* (bruchid on *Vigna radiata* and red gram), *Carposcapsa pomonella* (codling moth on walnut); Nematodes— *Heterodera scanchtii* (sugar beet cyst nematode), *H. humuli* (hop cyst nematode), *Rhadinaphelenchus cocophilus* (red ring nematode of coconut) and *Dithylenchus destructor* (potato rot nematode); Pathogens—*Fusarium oxysporum* (wilt of garlic), *Claviceps purpurea* (ergot of barley and wheat), *Botrytis cinerea* (gray mould of wheat, barley and grape), *Phomopsis phaseolorum* (pod and stem blight of soybean), *Puccinia carthami* (rust of safflower), *Ascochyta sojicola* (leaf spot and blight of soybean), *Uromyces betae* (sugar beet rust), *Fusarium nivale* (snow mould of wheat), *Ascochyta pinodella* (foot rot and collar rot of pea), *Ustilago tritici* (flag smut of wheat) and *Ustilago tritici* (loose smut of wheat and barley).

(iv) Germplasm Evaluation Characterisation and Documentation

NBPGR handles more than 75 major and minor crops at its headquarters and regional stations. The evaluation of indigenous and exotic germplasm over the years has resulted in the identification of several promising genetic stocks, resistance sources to diseases, and pests and nematodes which constitute important gene sources for crop improvement programmes.

The characterization and documentation of data has resulted in the preparation of crop catalogues/inventories on wheat, barley, amaranth, tomato, cluster bean, french bean, winged bean, cowpea, field pea, moth bean, soybean, lentil, *Sesbania*, *Trigonella*, Opium poppy, safflower, sunflower, sesame and maize (Paroda and Arora, 1986a). The establishment of computer facilities at the headquarters has recently accelerated the process of data documentation and cataloguing.

(v) Germplasm Conservation

Top priority has been assigned to conservation of genetic resources and NBPGR has recently built facilities for long-term storage of germplasm. Cold storage modules, currently operative at NBPGR, hold 76,000 accessions in different crops. A gene bank facility for conservation of over 2,000,000 accessions under long-term (−18°C) storage is being created presently.

NBPGR has also recently established a new facility for a plant tissue culture repository. This programme is to complement India's efforts in germplasm conservation. It lays major emphasis on *in vitro* conservation of clonally propagated plant species and cryo-preservation of pollen/recalcitrant species, as well as small seeded orthodox species in liquid nitrogen (−196°C). Research on monitoring of genetic stability using the isozyme system and cytological parameters are presently in progress.

Linkages

(i) Linkages with ICAR Institutes and Agricultural Universities

NBPGR has strengthened its linkages with several crop-based institutions/multicrop/regional institutions, project directorates, national centres and several All India-Coordinated Projects on different crops. The links with different agricultural universities in states and several ICAR centres located in these universities have equally important support and service from NBPGR in germplasm exchange, evaluation and field gene bank establishment. NBPGR also extends its support for conservation of base collections being held by any other institutions and provides facilities for data documentation, cataloguing, etc.

NBPGR continues to forge its strong linkages with crop-based institutions like the Central Potato Research Institute (CPRI), Shimla; the Central Rice Research Institute (CRRI), Cuttack; the Central Plantation Crops Research Institute (CPCRI), Kasargod; the Central Tuber Crops Research Institute (CTCRI), Trivandrum; the Central Institute of Cotton Research (CICR), Nagpur; the Sugarcane Breeding Institutes (SBI), Coimbatore; the Jute Agricultural Research Institute (JARI), Barrackpore; the Central Coffee Board, the Tea Experimental Station and several other commodity organisations, CSIR institutions and state department organisations. A few autonomous organisations also maintain direct links with NBPGR. The Institute for Horticulture Research for Northern Plains, Lucknow and the National Centre on Groundnut, Junagarh all have direct support in terms of germplasm exchange, collection and conservation of base collec-

tions of valuable plant resources. NBPGR also caters to the needs of premier institutions like IARI, New Delhi as well as the Project Directorate Wheat, New Delhi; the Project Directorate Rice, Hyderabad; the Project Directorate Pulses, Kanpur; and the Project Directorate of Oilseed Crops, Hyderabad. It also serves regional and problem-oriented institutes such as the Arid Zone Research Institute (CAZRI), Jodhpur; the Central Soil Salinity Institute, Karnal; and several others.

The national network in horticultural plants in India has received significant strengthening and expansion. This impetus primarily stems from the establishment of a full-fledged institute—the Indian Institute of Horticultural Research (IIHR) at Hassarghata, Bangalore in 1968 and its constituents centres working on different fruit plants. Prior to this, the Horticulture Division of the IARI and its few regional stations and eight other fruit research stations located in different agroclimatic zones were the only research centres engaged on research and improvement on a large number of fruit crops (Kaul, 1988). The situation has changed considerably in favour of horticulture. Presently there are five independent institutes, one National Research Centre and 12 All India-Coordinated Research Projects operating in 23 state agricultural universities on horticultural crops. Besides, ICAR Institute like IARI, NEH Complex, Shillong; the Central Arid Zone Research Institute, Jodhpur; the National Bureau of Plant Genetic Resources and its centres at Shimla, Jodhpur and Akola as well as Vivekanand Parvatiya Krishi Anusandhan Shalla (VPKAS), Almora; and Central Agricultural Research Institute, Port Blair; are also engaged in horticultural crops. State agricultural universities and regional centres are working on major and minor fruit plant species of regional importance.

(ii) Linkages with International Organisations

NBPGR continues to forge very strong links with several CGIAR-supported international institutes all over the world. During the past decade, there has been considerable strengthening of collaborative activities with IRRI, CIMMYT, ICRISAT, ICARDA, IITA, CIP, CIAT, AVRDC and also with IBPGR. Emphasis has been laid, apart from introduction and exchange of elite cultivars/strains, on evaluation of trial material and on development of scientific expertise. A joint collaborative programme for five years is in operation between NBPGR and ICRISAT, to carry out collection and evaluation of the ICRISAT mandate crops (sorghum, pearl millet, chick pea, pigeon pea and groundnut) and for minor millets. Untapped areas will be explored for germplasm collection, multilocation trials will be conducted and joint catalogues brought out. IRRI and CIMMYT have

extended their facilities for the training of scientists and joint collection expeditions were organised with IRRI to collect rice from India.

(iii) Linkages with IBPGR

The NBPGR maintains effective linkages with the International Board for Plant Genetic Resources (IBPGR), Rome, Italy. IBPGR, in the past extended support for conducting short training programmes in exploration and collection activities (1979, 1980 and 1982) and provided fellowship for training of scientists for full one-year and short courses at the University of Birmingham, England. The Bureau's expertise has also been availed at different times by IBPGR in germplasm collection in Africa and the Maldives. Recently, IBPGR-NBPGR undertook an expedition in Kenya, Sudan and Ethiopia for sorghum germplasm. IBPGR has recently signed a Memorandum of Understanding (MOU) with ICAR for better cooperation and coordination. IBPGR has also designated the NBPGR Gene Bank as the world repository for well over a dozen economic crop plants.

IBPGR has decided to establish its Regional Office for South and South East Asia at the NBPGR Campus, which will further strengthen these interactions. India has also extended its support to the F.A.O. Commission on Plant Genetic Resources and has given an undertaking for the free exchange of germplasm for research purposes.

Quarantine Regulations and International Exchange:

While realizing the importance of the exchange of seed/planting materials, it is also necessary to view critically the problems associated with exchange, particularly in view of the quarantine needs. Many crop species do not produce seeds and are propagated vegetatively. When these vegetative propagules are exchanged, the transfer of several associated diseases and pests cannot be overruled and thus could result in considerable risks, if stringent phytosanitary and quarantine regulations are not followed (Paroda *et al.*, 1987).

It is well recognised that hasty and uncontrolled introduction of germplasm material has the inherent risk of inadvertently introducing new pest/disease problems. This requires quarantine safeguards which may be divided in two groups: (a) legal safeguards and (b) functional safeguards.

(a) Legal Safeguards

Under the legal safeguards, import of seeds, plant propagules and other planting materials into India is regulated by the rules and regulations contained in the "Destructive Insects and Pests Act" (DIP Act) of 1914. This Act has been amended/corrected a number of times since then, the latest amendment being the plants and seeds (Regulation of Import into India) Order 1984, which became effective on June 24, 1985.

(b) Functional Safeguards

The Directorate of Plant Protection, Quarantine and Storage is basically responsible for implementation of quarantine safeguards in India and for this purpose, plant quarantine and fumigation stations have been established at all the international airports, seaports and land route stations. The incoming material is inspected, fumigated or otherwise disinfected before release. Quarantine greenhouse and screen house facilities are also available for postentry isolation growing at some of these stations. The stations under the Directorate handle bulk imports of commerce and seed/planting materials imported by private organisations/individuals. NBPGR, New Delhi is designated nodal agency of the Government of India for the exchange of germplasm material of all agrihorticultural and agrisilvicultural crops for research purposes. To fulfill its quarantine obligations, NBPGR created a separate Division of Plant Quarantine with its constituent units of Entomology, Nematology and Plant Pathology with laboratory facilities and experienced manpower. NBPGR has developed methods, procedures and techniques for detection and salvaging of imported seed and planting materials (Ramnath, 1987). A regional station at Hyderabad caters to the needs of plant quarantine in South India and also serves ICRISAT for its exchanges of germplasm.

Provision of Seed Act:

The Seed Act, 1966, Government of India provided the basis for regulating the quality of certain seeds for sale, and for matters connected therewith. The above act covers the whole of India and includes both agriculture and horticulture. It also specifies containers for the import of seeds; such as box, bottle, casket, tin, barrel, case, receptacle, sack, bag, wrapper or other things in which any article (seed/planting material) is placed or packed for import or export. The kind of article relates to one or more related species or subspecies of crop plants, each individually or collectively known by one common name.

Any person selling, keeping for sale, offering to sell, bartering or otherwise supplying any seed of a notified variety may, if he desires to have such seed certified by the certification agency, apply for the grant of certificate.

Required Modifications in the Quarantine Regulations:

The Indian Destructive Insects and Pests Act (DIP Act) does not have any specific recommendations or guidelines on the examination and release of such tissue-cultured materials. Hence, there is need to consider the following points for eventual modifications in the DIP Act based on experience gained on the subject.

(i) The consignment should be accompanied by an additional declaration that the material under tissue culture has been developed from tissues free from viruses, viroids, mycoplasma and fungi and bacteria (mentioned in the text).

(ii) After preliminary quarantine examination, it should be grown in a glass house/net house for one crop season to make sure that the plants developing from the plantlets are free from organisms which cannot be eliminated through tissue culture.

Besides these two suggestions, some other points which merit consideration for the inclusion in the DIP Act are:

(a) Germplasm material should be introduced in the form of seeds, unless vegetative propagation is necessary.

(b) For vegetative propagation, nonrooted material should be preferred over rooted plants.

(c) Consignments of vegetatively propagated material should be small. Each variety or species should be represented by a few tubers, cuttings or scions.

(d) If rooted material is received, cuttings derived from the roots should be released instead of the original roots themselves, which should be destroyed.

Breeders Rights and Commercial Linkages

The expanding horizons of patent rights in plant breeding is leading to a state of polarization in the field of conservation and utilization of plant germplasm. At the FAO Commission on Plant Genetic Resources in 1987, a proposal was made to institute a set of farmer's rights for the farmers in the "centre of origin" countries to

offset the impact of the breeder's rights in the developed countries (Swaminathan, 1988). According to him, this issue is gaining importance since genetic engineering enables us to transfer genes across several barriers. Wild species of economic plants occur primarily in the developing nations. Unless considerations of human welfare and sustainable development are coupled with considerations of commerce, competition and confrontation may replace cooperation in the plant sciences. He stressed that if this does not happen, the great leap forward in science and technology may result in a great step backward in international cooperation in the eradication of hunger and in the sustainable management of the biosphere and geosphere, including crop plant diversity.

More recently, several private national seed companies have emerged in the seed production business. A few of them also have an international network. Both kinds of commercial companies have been importing material from abroad (Table 1).

Future Thrust

The role of NBPGR in coordinating activities on plant genetic resources has increased tremendously over the past decade. To meet the growing demands of the breeders for elite material, cultivars and wild species and to share international commitments with the IARCs (IRRI, CIMMYT, ICARDA), the flow of genetic resources has been considerably high. Commensurate to this there is a dire need now to strengthen and create a plant quarantine infrastructure for ensuring quarantine safeguards involving the concerned crop institutes/projects, the Directorate of Plant Protection, agricultural universities and a few regional stations of NBPGR. This is particularly required for the postentry quarantine facility. In this context, an offshore quarantine facility is being established at the newly proposed station of the NBPGR at Andaman and Nicobar Islands. As for maintenance of working collections, national responsibilities are being defined and crop curators/institutions/centres are being identified. The responsibility of maintaining a base collection for long-term preservation of genetic resources will rest with NBPGR. Computer facilities for data documentation will also be generated at Bureau stations/other centres and with NBPGR providing the required coordination in this regard.

The highest priority in the national context is also attached to the establishment of an efficient facility for preservation of genetic diversity. The building of the National Germplasm Repository will be constructed near the present location of NBPGR with all modern equipment and gadgets, apart from the strengthening of present infrastructure, wherein the long-term storage modules installed have a

Table 1. Showing Import of Plant Material by Private Seed Industry.

S. No.	*International/ National Seed Company*	*Crop (No. of accessions/ varieties imported)*
1.	Cargill Southeast Asia Ltd.	*Helianthus* sp. (3 varieties)
2.	Coromandel Indag Products India (P) Ltd.	Tomato (15 varieties)
3.	Pioneer Seeds Co. Ltd.	Zea mays (10 samples), *Sorghum bicolor* (91 accessions), Sunflower (30), *Oryza sativa* (12)
4.	Mahyco Seed Company	Maize (405 accessions), *Triticum* sp. (195 samples) and Sunflower (4 varieties)
5.	Nath Seed Company	Tomato (23), Sunflower (40), Maize (9) and *Gossypium* spp. (7)
6.	Hindustan Lever Ltd.	New oil yielding species; Oil palm, *Macauba caryocar*, etc.
7.	Godavari Plywood Ltd.	*Eucalyptus* spp. (6), *Acacia* spp. (3), *Casuarina cunninghamia* (1) and *Sesbania formosa* (1)
8.	Tata Energy Research Institute	*Acacia* sp. (10), *Glincidia sepium* (37), *Oryza* sp. (14) and *Eucalyptus* supp. (5)
9.	Wimco Seedlings Ltd.	*Brassica oleracea, Lycopersicon esculentum* (27) Brassica oleracea (12)

capacity to store about 200,000 seed samples. Such facilities for medium seed storage are also to be generated at a few selected stations of NBPGR and other centres including crop institutes and agricultural universities. The Indo-U.S. Project proposed for strengthening the genetic resources activities has laid much stress and emphasis on the these aspects in generating/strengthening the necessary infrastructure, apart from training of personnel and visits of consultants/scientists both within the country and abroad for better and more effective linkages.

References

Arora, R.K. and Nayar, E.R. 1984. Wild Relatives of Crop Plants in India. *NBPGR Sci. Monogr. 9*: 90 pp.

Harlan, J.R. 1975. Crops and Man. Madison, Wisconsin, American Society of Agronomy and Crop Science Society of America.

Paroda, R.S. and Arora, R.K. 1986. Plant Genetic Resources—An Indian Perspective. *NBPGR Sci. Monogr. No. 10*: 34 pp.

Paroda, R.S., Ram Nath, M., Reddy, A.P.K. and Singh, B.P. 1987. Indian Plant Quarantine Systems for Rice. Proc. International Workshop on Rice Seed Health, held at International Rice Research Institute, Manila, Philippines, March 16-20, 1987 (In press).

Ram Nath, Mathur, V.K. and Verma, B.R. 1987. Plant Quarantine Activities in the Indian Context. Proc. National Symposium on Plant Genetic Resources, held at National Bureau of Plant Genetic Resources, New Delhi, March 3-6, 1987 (In press).

Vavilov, N.I. 1949/1950. The Origin, Variation, Immunity and Breeding of Cultivated Crops. *Chron. Bot. 13*: 364 pp.

Zeven, A.C. and de Wet, J.M.T. 1982. Dictionary of Cultivated Plants and Their Regions of Diversity. Wageningen, 259 pp.

Focused Cooperative Agricultural Research

A. Richard Baldwin
International Fund for Agricultural Research (IFAR)
Arlington, VA 22209

Introduction

The International Fund for Agricultural Research (IFAR) was established in the U.S.A. to stimulate interest in and support for international agricultural research. By this is meant conducting agricultural research by means of cooperative arrangements among public and private sector research institutions in developing and developed countries, including as appropriate the International Agricultural Research Institutes.

IFAR does not envisage being a "doer" of research or an active participating member of a particular cooperative research network or association. Because of its neutrality and relationship to the international agricultural research community it could, however, serve as a catalyst to stimulate cooperation. It can do this by providing a forum for discussion by interested parties, being a facilitator in the early stages of initiating a particular cooperative research venture, by helping to identify funding sources, and by raising incremental funds.

A novel mechanism is suggested for tackling agricultural research opportunities that are too large, too complex, too orphaned, or are considered impossible to achieve with the resources and procedures presently available to individual research institutions. The proposed mechanism and institutional arrangement provides focus, planning, coordination, and supplemental funding needed for "Manhattan" type research projects related to agriculture.

The basic difference between this mechanism and other research networking systems is that it provides a specific objective focus within a time focus as was done, for example, for the Moon Landing Program, i.e., within ten years send men to the moon and return them safely to Earth.

Numerous agricultural research projects with such focus are being considered, e.g.: (a) hybrid soybeans with 20% yield advantage by the year 2003, (b) practical biological nitrogen fixation for wheat by 2005, (c) cassava with 10 IU of Vitamin A equivalent per gram of cassava by 2003.

In this context IFAR has encouraged consideration of the concept described in this paper for focused large-scale agricultural research. The idea was first discussed with about 50 people responsible for large research programs related to agriculture. Everyone was enthusiastic about exploring the ideas further. IFAR then convened a group of 20 responsible scientists, who represented a large percentage of agricultural research in the U.S., to conduct a brainstorming session. The meeting was held 15 December 1987 at the World Bank in Washington, D.C.

After a full day of discussion, the group consensus was that the concept was indeed worth trying. IFAR was encouraged to take steps toward initiating at least two such focused cooperative agricultural research projects. This paper is a report of the proceedings of that December meeting.

The Concept

Most agricultural research projects, especially basic research, are of an ad hoc nature. Individuals or institutions seek funding for projects for which grant money is available and/or they feel interested in and capable of doing. The funding mechanisms and functional responsibilities have encouraged competition rather than cooperation. The results lead to diffused rather than focused research objectives and programs.

Circumstances are changing. Research funding is more limited. There is increasing clamor for faster adaptation of research results for economic and social gain. Several agencies have called for more focused research efforts, more coordination of research, and more networking of research programs. Tighter focus and closer cooperation are more likely to be achieved for the conduct of the upstream basic research than for the downstream applied research.

New scientific developments and technologies offer great opportunities in agricultural research that heretofore were not even fantasies. Biotechnology is here to be used to improve crop and animal production on a scale we have yet to appreciate fully. Recombinant DNA, gene transfer, plant regeneration, cell culture, monoclonal antibodies, and bioprocess engineering are tools to be applied to large and sophisticated projects that can offer great challenges to the best of our research scientists. Especially, it offers opportunities to increase sustainability and profitability of agricultural production around the world, particularly in developing countries where, despite recent advances in production (the Green Revolution), the needs are still enormous.

Some agricultural research opportunities are too large, too complex, too orphaned, or are considered impossible to achieve under our present system of research funding and competitiveness. There can be a mechanism to provide the focus, planning, coordination, and funding to tackle such complex projects. It would call for: (a) identification of a suitable worth-the-effort project with an objective and a time focus, (b) assembling a group of peers who are currently interested in and working on bits and pieces of such a project; they would represent various disciplines in both the public and private sectors, (c) identify the "state of the art," including who is doing what where, (d) the peer group to outline on a PERT-type chart what is essential to achieve the time-focused objective, (e) establishment of good communication and coordination among those working on relevant parts of the project, (f) identification of essential work that is not being done, and (g) securing supplemental funding so that all parts come together in an orderly way and build on each other to achieve the desired results.

There are precedents. The "Manhattan Project" and the "Moon Landing Project" were two examples with which we are familiar. In each, essentially impossible scientific and technical problems were overcome by integrating the activities of a diversity of individuals and organizations towards very clearly focused objectives. More recently the same approach is being taken in some medical and computer research projects. In the case of agricultural research, a range of institutions might be interested in participating in such coordinated programs, provided that appropriate mechanisms are in place.

Potential Participants

There are many *universities* with long-standing traditions regarding agricultural research—both basic and applied. Large amounts of federal and state funds have been provided for agricultural research over the years through, in particular, the Land Grant College System. Much good work has been done, but since the funds have been distributed on a relatively ad hoc basis rather than selectively deployed to meet predetermined goals, there has been no particular incentive for research institutions to cooperate. In fact, the reverse has probably been true. It is even difficult to get departments with different disciplines to cooperate in the same institutions. In the last decade funds have become increasingly difficult to obtain; this has sharpened competition to a point in some places where it has probably become counterproductive. Too much emphasis is now put on competing for resources rather than on collaboration to ensure more effective use of resources. Almost no time seems to be spent even on collaborative efforts to convince policymakers not to cut

allocations to plant sciences even further. The concept discussed in this paper offers an opportunity to create a climate in which such cooperation should be better able to flourish. This is not likely to be lost on the institutions involved once the concept is fully understood. It can be productive and exciting to be a part of a bigger whole.

Nor has it been customary for *agricultural research groups in the private sector* to coordinate their activities. To a considerable extent, this is because they have tended to concentrate their efforts on applied research whose purpose is to produce a product or process over which the companies wished to acquire proprietary rights. In such an environment competition precludes cooperation. However, the same institutions may well be prepared to work collectively on more fundamental problems, he solving of which will increase the sum of basic knowledge and lead to opportunities in which all parties involved will benefit. Since less and less funds are being made available from government sources for basic research in general and for basic research in the plant sciences in particular, the private sector will need to shoulder increasing costs (of basic research) that it has been able to avoid in the past. This will be expensive and corporations now have more incentives to cooperate. Their resources and advice will be vital to the kind of large focused research programs contemplated here. Under the proper circumstances such participation by the private sector can be very worthwhile and profitable.

The third group of potential partners are the *International Centers*. The Centers stand to benefit because such cooperative ventures would mobilize research capabilities and expertise that they cannot readily replicate and because such mechanisms would provide practical ways by which the Centers can serve as "bridge heads" between the "high tech" of the developed world and those in the developing world that need this technology but cannot readily develop it. The Centers also, of course, have much to offer—access to germplasm on a scale and in ways that nobody else can match, systems of field testing crops on a broad international scale, and a way to distribute results of certain types of research rapidly and internationally.

The *Federal agencies* are very interested candidates for participation in such cooperative projects. The USDA, in particular, has shown strong support for the idea and has made several suggestions for likely projects. The USDA, which is not only a funder but a doer of agricultural research, is very interested in networking, cooperating, focusing, and otherwise expediting their research toward recognized goals. Other agencies including the U.S. Agency for Interna-

tional Development, the National Science Foundation, and the Department of Energy have been helpful and encouraging. A representative of the Board on Agriculture has helped with exploration of the idea since its inception. The same is true for the Consultative Group on International Agricultural Research.

Potential Projects

Many long-range major research projects can be tackled only along the lines suggested if they are to be focused for objectives and time. Examples are listed below. Additionally, many other projects have been suggested but this list serves as a "starter." Each of these projects would fit the concept and have great worldwide economic and environmental significance for both producers and consumers.

1. Wheat with symbiotic nitrogen fixation to supply most of the nitrogen requirements by the year 2005.

2. Hybrid soybeans with 20% yield advantage by 2003.

3. High-yielding barley varieties that are tolerant to irrigation with brackish water (1.5% salt) by 2001.

4. Cassava with 10 IU of Vitamin A per gram of tuber by 2003.

5. High-yielding hybrid corn varieties that are tolerant to atmospheric temperatures of 25°F after germination by 2008.

6. Broad direct usage of true potato seed by 2000.

7. Rice varieties that have double the photosynthetic efficiency of current varieties by 2003.

8. High-yielding soybean varieties with less than 1% linolenic acid in their oil compositions by 2000.

9. Corn varieties with genetically engineered root worm control by 1998.

10. Introduction of sulfur-amino acid producing genes, possibly from Brazil nuts, into soybeans by 2003.

11. Corn varieties whose protein amino acid compositions are nutritionally balanced for swine and poultry by 2005.

Each of the above projects is being worked on by bits and pieces here and there. Also, very basic research, e.g., gene mapping and

biotechnology techniques, are being developed in several disciplines and will ultimately be important in the solutions to specific problems. A mechanism to replace and/or coordinate the current ad hoc approaches with more focused programs should greatly expedite achievements and probably facilitate accomplishments of goals that would not otherwise be possible.

The suggested projects serve only as examples to stimulate further thinking of what might be preferred projects in particular areas of your choice. However, the list above and many others are being considered by various groups. The projects can be made large or small and short- or long-term. However, to fit the model they must be focused as to a specific objective within a specified time frame.

Proprietary Rights, Patents, Licensing, and Royalties

Historically, these issues have severely inhibited opportunities for cooperation between public institutions and private corporations. It is particularly true in research programs that might lead directly and quickly to products or processes over which individuals or institutions wish to hold proprietary rights.

During the December 1987 meeting, this subject was discussed extensively. There have been changes in both the public and private sector approaches. For example, the USDA can now make exclusive licensing arrangements with a corporation for use of USDA patents. Such exclusivity can be bargained for either before or after the patents are issued. Furthermore, apparently, such exclusive agreements can be made even before the research is started. And USDA employees are encouraged to seek patents and to get them used.

Some of the universities now have patent development and licensing departments to handle patents more or less the same as the USDA indicated it could do at the December meeting. There seemed to be a general feeling that most universities would somehow be able to cooperate satisfactorily with the private sector in regard to this important subject. The proposal as presented and discussed called for working out satisfactory arrangements at the beginning of planning a cooperative project.

The private sector has participated in the discussion. The general feeling seems to be that "yes" they would like to be cooperators in such focused projects if they had a role to play and a clear arrangement is made ahead of time as to how they could use the results to produce and market new products. Certainly, participation of the private sector is important to any of the proposed projects. First,

their practical continuing advice and consultation during all aspects of a project is extremely important. Second, they can bring to the project great amounts of experience, professional scientific and technical capabilities, essential laboratory and field facilities, applied research and field testing expertise, and the ability to market and distribute the fruits of the cooperative research.

Implications

Since such projects have large potential for providing essential food and fiber for mankind's welfare and improving the economies of not only developed countries but also developing countries, they are very attractive. Payoffs should be substantial in every case.

For example, the first project listed on nitrogen fixation would conserve billions of dollars worth of energy from fossil fuels in an era (year 2010 and beyond) when such energy sources will become increasingly scarce and more expensive. Especially, this will be important in developing countries where nitrogen fertilizer is already expensive and difficult to obtain locally. Likewise, nitrogen fixing wheat would provide sustainability of crops at good yield levels—this is most important in developing parts of the world.

Similarly, let's assume that production of high-yielding hybrid soybeans can be achieved in 15 years. Yields should increase 15 to 25% with enhanced economic values aggregating several billion dollars per year. The crop should be more secure and have broader adaptability. Another project relating to soybeans is the insertion of genes for increasing methionine/cysteine contents. This would significantly improve the nutritive value of soy proteins for humans and for animals raised for meat, milk, and egg products. Increased values of soy products would total hundreds of millions of dollars per year.

One of the significant debilitating diseases with loss of human productivity in many developing countries (especially in the tropics) is caused by lack of sufficient Vitamin A in the diet. A main source of calories in many such tropical areas is cassava. A project to introduce Vitamin A producing genes into cassava could alleviate much of this nutritional problem and improve human productivity considerably. Likewise, changes in the nutritional contents of all food crops will have even wider implications.

Crop plants are essentially poor converters of solar energy to usefulproducts—they utilize only 1-2% of the solar energy available. Accomplishment of project 7 above with all the fallout implications for other crops would provide additional food and

fiber for several additional billions of people on this earth. It would reduce other energy demands, improve sustainability of agricultural production at significantly higher levels, and would provide economic benefits almost unimaginable.

If we can solve the riddle of cold tolerance, e.g., for oats, and transfer that capability to corn (or soybeans) as suggested in project 5 above, imagine the benefits to be achieved by thus providing longer-growing seasons. Similarly, increased salt tolerance in a major crop such as barley (or some other crop) would relieve one of the major problems with irrigated crops and extend possible crop production to brackish water areas where no food crops can be grown today.

It will take many years to accomplish some of these projects even with the proposed more focused cooperative approach. Furthermore, as results are expedited on one project, many fallout techniques or products can become available to other related projects. The world demand for food continues to increase daily as newcomers are to be fed. The capacity of traditional approaches for agricultural research to increase crop yields by 2-3% per year seems to be declining. New approaches and new research management linkages are necessary if we are to provide sufficient safe and high quality food for future generations. There will be billions more people, and the question is not whether they will be fed but how well they will be fed.

It is perceived by many that the proposal discussed here has a good chance to expedite agricultural developments at lower cost. We should not miss this opportunity!

Initiation and Selection of a Project

Although details for an organization to implement such projects must be project and group-specific and be worked out collectively with and by the collaborators concerned, a start on the overall process might be as follows:

Assuming that an IFAR-sponsored meeting, such as that held in December 1987, might identify the kind of problem that could be resolved through a focused cooperative research project, the topic would then be made known to whomever seemed most likely to be interested in participating. Their views would be solicited and if the response justified it, a meeting would be held, under the sponsorship of IFAR, to examine the proposal in more detail. If it looked to be a feasible project with the right kind of support, a small initiating group would be set up to commission a state-of-the-art

review to provide the framework for the project. This review would also identify who is doing what, where, and how they are currently being funded. This working group (not IFAR) would then invite potential collaborators to participate. Probably 20 or 30 scientists/institutions, both public and private, might thereby become involved.

Modus Operandi

Methods of operation will, of course, differ for each project. Procedures for selecting, planning, and operating a model project might be as shown below:

A. The project would be identified as described above and a small working group chosen to initiate it.

B. The working group would then convene and commission an extensive review of the subject in such a way that it would identify requirements for the project to be successful and who is already doing what that is relevant to the project as a whole. Current resources available to potential collaborators would be estimated. The review would also include a draft protocol which would determine how the network would operate.

C. The working team would then consider the review report and, assuming the project is still a "go," would invite other relevant participants to join in providing the network of basic and applied scientists. Since this would be a voluntary collaboration, participants could drop out or new ones could be added at any time.

D. This group as a whole would choose a "leader" or "chair" to oversee the project and to act as a secretariat and/or coordinator.

E. The network participants would each identify his or her part of the project and its current source of funding. It would not be expected that additional funding would be used to replace current funding.

F. The network participants would identify research that is not ongoing, but is essential to the success of the project. The team would identify scientists who could work on the missing links and recommend the additional funding requirements to IFAR. IFAR would try to identify sources of such funding and/or help secure the funding.

G. The participating team early on would establish its own rules for patents, licensing and royalties. There are many ways to handle this within the general parameters discussed under the section above on this subject. Some suggestions have been as follows:

1. All patents could go to the inventors or their institutions with exclusive licensing to the project for sublicensing to the private sector participants. Royalties would flow back to the inventors. Or,

2. All patents and rights to be assigned to the project with exclusive licensing to the private sector participants—or whoever is going to provide the marketing and/or distribution. All royalties to go to the project, to be distributed by the management team or entity to the inventors. The project team could possibly form a corporation to do this.

At the December 1987 meeting there seemed to be a predominant sentiment for Option 1 above. All agreed that it is very important to have comfortable consensus on this and work out the details early in the planning and organizational process.

H. The project secretariat would provide communications among the participants by personal contacts, periodic conferences (at least annually), and some form of newsletter. Funding for such activities might be identified by or raised by IFAR.

I. The periodic conferences would provide a means of: 1) keeping all participants up-to-date on the progress of each subpart of the project; 2) providing critiques and advice in the form of peer review; 3) guiding the overall program toward its goals; 4) identifying additional essential research and recommending such for funding: and 5) providing a general cohesiveness among the collaborators.

J. The private sector participants would be an important part of the project from the beginning. They would provide advice, counsel, and experience in addition to their portion of the work on either basic or applied research as indicated with the objective of moving the process toward "commercialization" as soon as practical.

K. If distribution of the project results is to be via public institutions, such as State Experiment Stations or the International Centers, they would participate and fulfill their roles by applied

research, field testing, selection, adaptation, and release of material as expeditiously as possible.

L. This "Marketing and Distribution" by either the private or public sector is the key objective of the project. These projects are for the purpose of achieving useful results, not just adding to the literature. However, of course, most of the research results during the course of the project will be published or otherwise distributed. This should generate additional by-product or spin-off ideas and products.

Funding Requirements and Sources

Research of the kind envisaged is likely to be expensive, albeit very cost-effective. Also, these are long-term programs of 10 to 15 years' duration. Therefore, most of the funding requirements will be project-specific and be attractive most likely to those most interested in achieving the results. As mentioned before, it is not anticipated that new funding will be required for ongoing research that becomes a part of the network. New funding probably would be required for essential expanded and/or newly initiated research.

Discussions with various people that might be responsible for such funding have seemed to be enthusiastic about the possibilities. Comments such as: "This is a very salable concept." or "This is a merchantable idea." or "There is a good possibility that funds can be diverted gradually from other phaseout projects to such a program."

IFAR can play a role in helping to identify funding sources and assisting in obtaining the necessary funds, without becoming involved in the science or administration of a project.

The Next Step

It would appear that sufficient exploratory discussions of the concept of Focused Large-scale Cooperative Research Programs have been made. The conclusion of the December 1987 meeting was that the process should be continued with the objective of trying to initiate at least two such projects. Therefore, IFAR suggests that specific proposals be put forward by interested parties such as USDA, NSF, USAID, the International Centers, universities, the private sector, and appropriate groups of researchers. Preferably such initial proposals should identify the project, interested participants, and specific objectives. This information should be sent to the International Fund for Agricultural Research, 1611 North Kent Street, Suite 600, Arlington, VA 22209.

CIMMYT'S Initiative in Biotechnology and the Initiation of an International Collaborative Network on Maize Biotechnology Using Molecular Markers—Role of the Private Sector

Clive James
Deputy Director General for Research, CIMMYT
Mexico

Introduction

Madam Chairperson, ladies and gentlemen, I very much welcome this important opportunity of sharing with you some views on new approaches for collaborative research in biotechnology between the International Agricultural Research Centers (IARCs), particularly CIMMYT, and the private sector. More specifically I wish to share with you some of the general information on CIMMYT's proposed future program in biotechnology and an international collaborative research network on maize biotechnology using molecular markers, that CIMMYT is attempting to initiate with private and public institutions. At the outset I wish to emphasize that the views I will present this morning are my own views and do not necessarily represent those of CIMMYT as an institute. I would also like to acknowledge the important inputs to the Collaborative Network on Maize Biotechnology by two persons who are attending this conference. Dr. Ron Phillips from the University of Minnesota, whom CIMMYT has retained as a consultant on biotechnology. The other is Dr. David Jewell, a CIMMYT maize scientist, working on the introduction of alien genes to maize and currently on a study leave at Minnesota with Ron Phillips working on restriction fragment length polymorphism (RFLP) applications. Ron Phillips and Dave Jewell have provided the technical inputs into the collaborative project and will be prepared to respond to specific questions of a more technical nature. In my role as a scientific administrator, I will cover the rationale for CIMMYT's initiative in biotechnology, the conceptual framework and the organizational aspects of the network. I would also like to address the issue of why I believe that such collaborative projects in the area of biotechnology between CIMMYT and the private sector are not only desirable, but essential if we as a global agricultural research community are to utilize limited research resources in the most cost-effective and responsible way.

In addressing this important topic in 30 minutes I will of necessity select only certain themes and consequently there will be important issues that will not be treated. So let me share with you now the

themes that I will attempt to cover this morning so that you are aware of the outset of the broad framework within which particular issues will be discussed in greater detail.

First I will review some experience of the past because this is often one of the best indicators of what may be possible in the future. More specifically I will briefly cover the evolving role of the respective contributions of public and private sector institutions to agricultural research in the industrial countries, the donor countries to the CGIAR—because I believe there are many important lessons to learn from this experience.

Secondly, I will share with you my assessment of the changing role of the international agricultural centers during the next decade and beyond. In particular I will comment on the rationale for the establishment of a biotechnology unit at CIMMYT, and its proposed program thrusts, and the vital importance of networks for facilitating collaboration between public institutions and the private sector in biotechnology.

Finally I will discuss, as a specific example, the conceptual framework, organizational strategy and status of the International Collaborative Research Network on Maize Biotechnology that CIMMYT has initiated, and which involves both public and private sector institutions in Europe and the developing countries, and which specifically addresses the use of RFLPs as molecular genetic markers in maize.

CONTRIBUTIONS OF PUBLIC AND PRIVATE SECTOR INSTITUTIONS TO AGRICULTURAL RESEARCH

During the twentieth century, in many, if not most, of the industrial countries there has been a marked shift in the respective division of labor between public and private sector contributions to agricultural research, including plant breeding. Initially, public institution breeders developed and released varieties of crops like maize to farmers directly. Later, seed companies started to get involved with seed distribution to serve a growing and wider community of farmers. However, over the last twenty years, there has been a marked shift in many industrial countries, which has resulted in plant breeding activities and corresponding research shifting from the public sector to the private sector. Several factors have been responsible for this shift, including shortage of funds for public sector institutions, legislative changes such as patent laws and plant variety rights, which have encouraged private sector involvement; and I think it is important to acknowledge that these changes have been facilitated indirectly and directly by national governmental

policies. The most recent graphic example of the direct effect of governmental policy to shift plant breeding activities and corresponding research from the public to the private sector was the privatisation in 1987 of the Plant Breeding Institute at Cambridge, England.

This shift in emphasis has very important implications as far as biotechnology is concerned, because this has led to a situation where significant investments in research on biotechnology, particularly on hybrid crops like maize are now made by corporations in the private sector. These are cost-effective investments for the corporations, particularly the transnationals, because of the economies of scale associated with their global operations. Furthermore, shortages of public funds and encouragement through national policies have led the private sector to fund an increasing proportion of research on biotechnology in public institutions on a contract basis. It is noteworthy that the value of these biotechnology contracts are usually much higher than the traditional collaborative research contracts because of the high cost of conducting research on biotechnology. Thus, in many of the premier public institutions the biotechnology contracts represent an increasing percentage of their total contract portfolio.

We may conclude that the private sector has played a significant and increasingly important role in agricultural research in the industrial countries and currently are often significant investors in basic/strategic research on biotechnology applications that can contribute to the increased efficiency of plant breeding It is also important to recognize that the increased participation of the private sector has been facilitated indirectly or directly by the policies of governments which are also donors to the CGIAR. If we accept that the private sector has played a significant role in increasing productivity in the industrial countries, then it may be reasonable to suggest that similar benefits may accrue to the developing countries if efforts are made to promote private sector involvement more vigorously in the Third World in activities where they may have comparative advantage, like biotechnology. And there are other areas like seed multiplication, production and distribution.

As far as biotechnology is concerned I believe that the involvement of the private sector in collaborative research networks, facilitated by the CGIAR, is very important particularly for a crop such as maize in which the private sector is already investing significant resources in biotechnology.

CHANGING ROLE OF IARCs: UPSTREAM RESEARCH AND COLLABORATIVE NETWORKS

The CGIAR and its precursor founding foundations, Rockefeller and Ford, have provided the very important feature of institutional continuity in applied agricultural research for the developing countries. As contrasted to most bilateral and multilateral agricultural research projects, the CGIAR system has provided focus through a multidisciplinary approach and perhaps the most important element that has contributed to success is the unique continuity provided in terms of purpose, programs and people. The best example of this is the work of Dr. Norman Borlaug who provided innovative direction and continuity to the wheat program in Mexico over a forty-year period. Agricultural development is a long-term venture and agricultural research requires even a longer term commitment. Hence, continuity is not only desirable, but essential if the investment that we are going to make in agricultural research, including biotechnology, is going to be cost- effective. The clients of the CGIAR system are the national programs in the Third World and the principal objective is to assist national programs to develop and utilize research products and services that will result in increases in crop productivity and efficiency. To date, the CGIAR system is acknowledged to have been one of the most cost-effective investments in international agricultural research. One of the roles the CGIAR system has played is that of the apolitical broker that has facilitated the generation, transfer and adaptation technology from the industrial countries to the developing world. If we accept that the system has had a measure of success in some developing countries as a result of building capacity in national programs, it follows that the future research agenda of the system should change to some extent because the client countries, with improved capacities, can now implement some of the activities that were initially performed by the centers:

It is noteworthy that the CGIAR is only one very small, albeit effective, component in the complex of international agricultural research, accounting for only 3% of public resources devoted globally to agricultural research. Therefore, in order to utilize its limited resources in the most effective way, the system must continuously monitor the dynamic research environment and identify the type of research where it has comparative advantage and reallocate its limited resources accordingly. The CGIAR system has used the following categories to define the different types of research. Like all classifications, it is arbitrary but it is useful in distinguishing the different areas of research and the respective roles of various institutions.

1. *Basic research* is that designed to generate new understanding (e.g., studying the mechanism of resistance to a pest).

2. *Strategic research* is that designed for the solution of specific research problems or the development of a technique (e.g., the technique for detecting dwarfing genes in wheat seedlings).

3. *Applied research* is that designed to create new technology (e.g., breeding new varieties of dwarf wheat that can respond to high levels of nitrogen without lodging).

4. *Adaptive research* is that designed to adjust technology to the specific needs of a particular set of environmental conditions in a specific region (e.g., adapting dwarf wheats with earlier maturity for farming systems in Bangladesh).

These different types of research are part of a continuum: "upstream" is used to mean a movement towards basic research and "downstream," a movement towards adaptive research.

Traditionally, the CGIAR system has conducted mainly applied research, but in the view of the Technical Advisory Committee (TAC), which monitors technical developments at the system level, the centers should move upstream by increasing emphasis on strategic as opposed to applied research. The TAC argues that the centers must move upstream if they are to retain their comparative advantage in the future and also to complement the changing needs of those national programs which have increased their capability in applied research and are requesting more assistance from the centers in terms of strategic research and particularly biotechnology. The TAC has also placed a high priority on the application of biotechnology techniques because this may allow the centers themselves to increase the efficiency of their own current breeding programs.

CIMMYT has considered the TAC recommendations and responded to the TAC priority on biotechnology by deciding to establish a modest biotechnology laboratory at CIMMYT which will hopefully be completed by the end of 1988. The laboratory will serve both the maize and wheat programs and funds are already in place to recruit two senior molecular biologists/geneticists to be augmented by visiting scientists, associated scientists and postdoctoral fellows to provide a minimal critical scientific mass. I believe that CIMMYT, as an institute focussing on applied research for crop improvement, provides an ideal and fertile forum for locating such a biotechnology unit. Indeed, I think the strategy of locating biotechnology units within the centers is critical and will allow us

to increase the harvest index from biotechnology by facilitating the dialogue between the biotechnologist and those involved in germplasm development—if this communication gap is not effectively bridged, it will result in a loss for everybody concerned, including researchers, producers, and consumers.

The focus of the biotechnology program in CIMMYT will be general in nature and will include the utilization of diagnostic techniques including monoclonal antibodies and molecular probes. It will also explore in the near term the use of RFLPs as genetic markers, concentrating on traits that are not easily managed in the current breeding programs. It is imperative that the program thrusts of the unit be problem oriented and seek to develop biotechnology techniques that will increase efficiency and augment the current techniques used in the breeding programs.

Another important feature is that CIMMYT will not seek to *develop* biotechnology techniques *per se*—this we believe to be the comparative advantage of public and private sector institutions involved in more basic/strategic research in the industrial/donor countries—CIMMYT's major role will be to *test, adapt* and *utilize* biotechnology techniques and applications that have been developed by collaborators and, if appropriate, incorporate the new techniques in the current breeding programs. Ultimately CIMMYT should transfer appropriate techniques to the developing countries by training national program staff at its lab in Mexico and the labs of collaborators. This means that the *modus operandi* of the biotechnology program will be collaborative in nature and it must be networked with other biotechnology centers in both the industrial and developing countries. To practice good science, one must have a minimal and critical level of direct involvement in research. Therefore, in order to be meaningful participants in this network, we believe that it is critical that CIMMYT have a biotechnology laboratory on site and a minimal critical mass of internationally recognized staff in biotechnology that will facilitate a meaningful dialogue, and provide the center with an in-house, hands-on capability in biotechnology. This will allow CIMMYT to contribute to a network and become active members of the international biotechnology community and be in a position to judge the merit and value of potential techniques in the international marketplace. Assessing the value of biotechnology products will probably become an increasingly important requirement in the future, when the centers may well be engaged in purchasing the products of biotechnology from a private or public sector institution, for example a gene controlling an important trait.

Collaborative research with public institutions in the industrial countries is not a new idea to the CGIAR system and CIMMYT currently has a portfolio of approximately 90 such projects with centers of excellence in North America, Europe and Oceania. The two features that I believe do merit emphasis in the new Biotechnology Collaborative Program is firstly, close contacts with the private sector and secondly, more recognition to the important role that the centers can play as facilitators of international networks.

Networks are now being favored as an effective mechanism of implementing and coordinating international agricultural research. In a world that is becoming increasingly interdependent, networking has many merits but it is not a panacea. In essence a network is a working group of individuals or institutions who have a common goal and who share information and plan or implement a joint working program. There are three types of networks. The first type involves only the exchange of information and the element that distinguishes the second type is the addition of meetings to facilitate information exchange. The third type includes the elements of the first two plus the important addition of joint priority setting, planning and division of labor that reflects the comparative advantage of network members and that creates interdependence amongst participants. Whereas all three types have merit, it is probably the Type 3 network that I think we should strive for in an International Collaborative Network on Biotechnology in order to optimize the use of international resources in biotechnology by avoiding duplication and accelerating progress towards common goals and objectives.

AN INTERNATIONAL COLLABORATIVE NETWORK ON MAIZE BIOTECHNOLOGY

In late 1986, CIMMYT in conjunction with its biotechnology consultant Dr. Ron Phillips of Minnesota, initiated action to develop a proposal for the establishment of an International Collaborative Network on Maize Biotechnology that would involve public and private sector institutes from both the industrial and developing countries. As an international centre with the global mandate for maize, it is appropriate for CIMMYT to play the role of a facilitator for such an international network and a special effort was made to involve the private sector which currently is a significant investor in maize biotechnology. Discussions started during a meeting held in Bad Homburg, Germany in August 1986 where the research directors of the IARCs were asked to share with their counterparts in the European donor countries the future research agendas on strategic research, including biotechnology, that the IARCs would like in-

stitutes in Europe to undertake as part of a collaborative research program. The private sector in Europe was represented at the Bad Homburg meeting by Dr. Joseph Seitzer, Research Director of KWS, Einbeck, Germany. I would like to take this opportunity to acknowledge CIMMYT's appreciation to Dr. Seitzer and the excellent cooperation of KWS, which has allowed us to proceed with the negotiations to establish an international network of private and public institutions in Europe.

Before giving you a general and brief description of the proposed network, which is still in its initial stage, let me preface my remarks with some principles that have guided the approach to forming the network.

1. Developing collaborative research arrangements, and in particular networks, is a very complex process and prudency would suggest that if we want to increase the probability of success we should make haste slowly.

2. It is important that the project be embarked upon within the context of a pilot project. This allows the flexibility to involve new partners, for periodic reviews, and if necessary, a change in procedures during the course of the project.

3. Acknowledging that there are new and potentially difficult problems related to intellectual property rights and patents, it would be desirable to initiate a collaborative network with the private sector on research of a more generic nature, so that the partners in a network can first get to know each other, and build mutual trust, confidence, and professional respect. The building of trust amongst partners is a key prerequisite step to negotiating more complex agreements related to property issues. Indeed, I believe it may be counterproductive to try and resolve complex issues related to intellectual property ownership in a vacuum at the outset of the project, without having the benefit of working together on a pilot project which can act as a testing ground and reveal some of the key issues which may be difficult to anticipate, and in all probability, likely to be case-specific.

The strategy I am suggesting is a cautious, step by step process which provides flexibility and an opportunity for trust to be developed and the requirements of the different parties to be respected and negotiated.

MAJOR OBJECTIVES OF THE CIMMYT/RFLP MAIZE NETWORK

CIMMYT is seeking to explore and facilitate the possibility of establishing an international network of public and private research

groups with specific interest in the application of molecular genetics to maize plant breeding. We sense that similar efforts are being pursued at various locations in developed countries and believe that a network structure would be useful for coordination and distribution of information.

Interest among practical plant breeders to employ molecular markers as a selection tool has increased recently. Several publications indicate that RFLPs could improve efficiency of selection for qualitative and quantitative traits. However, precise information about their practical application is still lacking. Analytical techniques need to be developed and adapted for large scale analyses, and the molecular data related to field data. The network participants would collect and analyze relevant data, and exchange information. This should accelerate progress and make efficient use of limited resources.

The major goal of the network is to explore the feasibility of employing molecular techniques to aid selection work, and thus make maize breeding more efficient. The RFLP molecular genetic markers will be used to determine linkages with genetic factors controlling quantitative trait expression. Upon finding associations between the molecular genetic markers and quantitative trait loci (QTLs), we then want to determine how to best utilize the major linkages in maize breeding programs.

CIMMYT recognizes the long-term nature of this research and the need to share information on the difficult task of applying molecular genetics in plant breeding. Ultimately CIMMYT would hope to be in a position to advise developing countries on useful and appropriate molecular genetic technologies.

Availability of Probes

Through the generous offices of Dr. David Hoisington of the University of Missouri, a suite of RFLP probes has been donated for the use of public and private sector network members. The suite comprises over 100 mapped probes on all 20 arms of the maize chromosomes. The use of a common set of probes will facilitate the integration of information and analysis of data.

Traits of Interest and Division of Labor Amongst Network Members

Initial emphasis would focus on quantitative traits that are not easily selected for in current breeding programs or expensive to screen under field conditions. These traits include factors such as insect resistance (e.g., corn borer), stalk strength, drought tolerance,

daylength insensitivity, cold and heat tolerance, aluminum tolerance, and traits introduced through alien genes. CIMMYT would also be interested in developing probes for traits that cannot be screened in Mexico, e.g., maize streak virus, a pathogen that is not endemic in Mexico. Whereas there is obviously great interest in yield as the important trait, there is also recognition that this is a very long term objective.

Division of labor amongst network participants would be on the basis of a specific trait of mutual interest to the network participants. Each participating lab or group would have the responsibility of generating information relative to one trait of interest and sharing the data with the other participants. Thus, the attractive feature of the network is that it provides an incentive for each member, because each network participant will receive information on several traits in return for sharing information on one trait.

Selection of Maize Germplasm/Lines

Each network participant would select 40 lines of proven performance for a specific trait. The lines used by the network will contain at least 50% of public lines. Private lines can also be nominated by network members.

Analysis of Data, Development of Data Base/Sharing of Information

Initially, individual members will analyze their own data utilizing techniques that will be developed, acquired and consolidated within the network over the course of time. There are obviously advantages to centralizing some of the information analysis and this will be done as the work progresses. Information will be shared freely amongst the network members and publication will be encouraged. As the network develops, CIMMYT will facilitate the exchange of information and plans to build a data base that would be accessible to the global scientific community; the latter will require significant effort, but is critical to the operation of an effective network and to ensure free access to the consolidated data base which will capture the outputs of the network.

Clone Repositories and Training

CIMMYT will encourage and try to facilitate funding for repositories of the various mapped RFLPs of maize and other specific probes at more than one location; in choosing locations consideration will be given to freedom of access, comparative advantage and cost-effectiveness. The responsibility of the repositories would be to collect, verify, store, publicize availability, and distribute clones.

CIMMYT would particularly facilitate the availability of clones to interested laboratories in developing countries.

As appropriate techniques are developed, training in their utilization will become important, and CIMMYT would encourage temporary transfer of visiting scientists between network laboratories and place high priority on training national program scientists at CIMMYT and in network laboratories.

Current status of the network

Discussions have proceeded over the last year or so and the table below shows the private and public institutions involved from four countries in Europe— Germany, France, Holland and Italy.

European Institutions Associated with Maize RFLP network

Private Sector	Public Sector
Germany	
KWS	Max Planck, Cologne University of Munich University of Hohenheim
France	
LIMAGRAIN	Laboratorie GPDP of CNRS Gif-sur-Ivettee
Italy	
AMI	Gergamo Institute
Holland	
Van der Haave	SVP (Wageningen)

The proposed European arm of the network can be associated with a larger international network which could be developed into a unique international collaborative research network. This should further facilitate operation of joint research programs and the transfer of technology between the private and the public sector and the industrial and the developing countries. The European group has already agreed to submit a two-phase, five-year proposal to EUREKA, the pan European funding organization funded by the ministries of science and technology, in the respective European countries. All four European countries have already submitted their applications and judging from initial responses, the proposal has a reasonable probability of being approved in the imminent future. However, it is important to note that funds for the initial project ac-

tivities have already been pledged by some of the private sector institutions and personnel have been recruited to conduct some of the experimental work in 1988.

CIMMYT, as the initiator, is a full partner in the network and has already selected the required 40 maize lines with various levels of resistance to Southwestern Corn Borer (*Diatrea grandiosella*). Facilities for conducting the DNA extraction and analysis are being incorporated in the new CIMMYT lab, but the 1988 analyses may have to be conducted at another network facility if the CIMMYT lab is not ready by year end.

From the outset, CIMMYT has given high priority to associating developing countries with the network. This was first facilitated through CIMMYT's role in developing a proposal with CINVESTAV—the Advanced Institute on Biotechnology based at Irapuato, Mexico, on Maize RFLPs which is already operational and funded by the Rockefeller Foundation. Also through the efforts of Dr. Ricardo Magnavaca from EMBRAPA, Brazil has already indicated its intention to join the network, and will probably select aluminum tolerance/phosphate efficiency as the specific trait.

Institutes from Developing Countries Involved with the Maize RFLP Network

CINVESTAV, Irapuato, Mexico
EMBRAPA, Sete Lagoas, Brazil

In an effort to share information on the status of the European network with colleagues in the United States and to explore interest in initiating a similar network in the United States, Dr. Ron Phillips co-organized with CIMMYT an informal meeting, hosted by Agrigenetics at Madison in February of this year. Representatives of several of the major public institutions working on maize breeding/molecular biology attended and were sponsored by USAID; staff from Agrigenetics and Pioneer attended from the private sector. I would like to thank USAID, and Dr. Brady in particular, for the opportunity today to share with you more formally CIMMYT's proposals for biotechnology and the status of the network. It is my sincere hope that U.S. public and private institutes, which represent the world's major source of knowledge on maize, will be able to participate in a network that will allow the global scientific community and maize producers and consumers in the industrial and developing countries to benefit from your expertise and the advances in molecular biology

The Rockefeller Foundation's International Program on Rice Biotechnology

Gary H. Toenniessen, Associate Director
and
Robert W. Herdt, Director
Agricultural Sciences Division, The Rockefeller Foundation

Introduction

The goals of the Rockefeller Foundation's International Program on Rice Biotechnology are: 1) to assure that new techniques for crop genetic improvement based on advances in molecular and cellular biology are developed for rice, 2) to facilitate the transfer of these biotechnologies to rice breeding programs in the developing world for use in producing improved varieties that address priority needs, and 3) to help build the scientific research capacity necessary for continued development and application of new rice genetic improvement technologies in selected developing countries.

The Foundation's Trustees approved the program in December 1984 with the realization that a 10 to 15 year commitment of $3-5 million/year might be required. Research at several laboratories began in 1985, and others have been added as the program has evolved and expanded. Over 50 projects are now supported with collaborative research conducted by scientists from throughout the world. Further expansion of the program in developing countries is anticipated. The program evolved from earlier Foundation work in agriculture and was designed keeping in mind principles learned from earlier programs.

Building on Solid Foundations. The International Program on Rice Biotechnology builds upon and is a continuation of the Foundation's long-term commitment to promote more effective use of science and technology to improve food production and living conditions in the developing world. As early as 1913, the Foundation's International Health Board supported research to increase agricultural productivity in China as a means of improving the nutritional status of the population.

From 1932 to 1959, the Rockefeller Foundation committed nearly $50 million to research in experimental biology. Under the direction of mathematician Warren Weaver, that program helped to bring the tools of mathematics, physics, and chemistry to the study of living organisms. This early research in biochemistry and biophysics was critical in laying the foundations for modern

molecular genetics. Dr. Weaver has even been credited with coin-i..g the term "molecular biology" (Kohler, Robert E., 1976 *Minerva* Vol. 14, No. 3, pp. 279-306).

The Foundation's major international field research programs in agriculture began in Mexico in 1943 under the leadership of J. George Harrar. Initially, the research was diverse and expansion occurred on a country-by-country basis. As part of the Mexican program, high-yielding, semidwarf wheat varieties were developed by Norman Borlaug and colleagues. When tested in India and Pakistan, the Mexican wheats performed well and were widely adopted by farmers. Thus, it was shown that improved seed could provide an effective mechanism for delivering the benefits of science and technology across broad regions of the developing world.

To apply this same strategy, often referred to as a germplasm-based technology, to additional crops and additional countries, the Rockefeller Foundation joined with the Ford Foundation in establishing the International Rice Research Institute (IRRI) in the Philippines, the International Center for Maize and Wheat Improvement (CIMMYT) in Mexico, the International Institute for Tropical Agriculture (IITA) in Nigeria, and the International Center for Tropical Agricultural (CIAT) in Colombia. The accomplishments of these institutions have been impressive. High-yielding rice and wheat varieties, for example, are now grown on over 55 million hectares—or one-third of the area planted to these cereals in the developing countries. Funding for these international agricultural research institutes and several others is now provided by many donors and coordinated by the Consultative Group on International Agricultural Research.

Despite the success of these institutes, it became apparent in the 1970s that their research output often did not flow automatically to national programs. To assist national agricultural research programs in using institute technologies and in generating their own, the Foundation established the International Agricultural Development Service in 1975. In 1984, IADS was merged with two other organizations to form the Winrock International Institute for Agricultural Development which, now completely independent of the Foundation, continues to provide services to help strengthen national agricultural research programs.

In 1982, a major review of the Foundation's agricultural program was undertaken with the assistance of external consultants. These advisors praised past accomplishments and recommended that the Foundation's agricultural program be continued but restructured

around three new thrusts. One would be a major commitment to research on the application of molecular biology to plant breeding in developing countries. This was a logical sequel for the Foundation's agricultural program, building upon previous investments in both fundamental biological research and in the development of a research capacity for crop genetic improvement in the developing world. The Foundation's trustees approved these recommendations, and the staff began the process of designing the program.

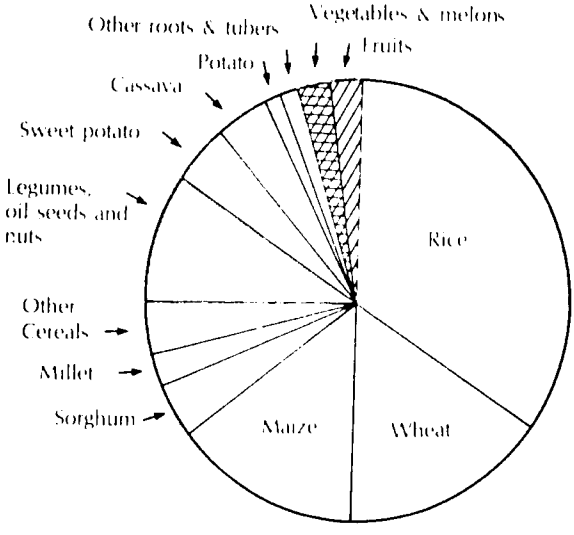
Focus on Rice. In selecting a focus for a program that would apply molecular biology to plant breeding and direct the benefits to the disadvantaged in developing countries, Foundation officers sought: 1) to identify an important need that was not being addressed by others, 2) to attain a high degree of synergism amongst the various activities to be supported, and 3) to have a strategy which would allow for eventual Foundation disengagement. Three types of options were given serious consideration. One, suggested by the external review team, would have provided support for crop-focused programs at each of several selected universities. Each university would combine molecular biology with plant breeding for its selected crop and establish linkages with the appropriate international agricultural research institute.

A second option would have provided support for research conducted collaboratively by developed country and developing country institutions on the molecular genetics of agronomic traits uniquely important for crop production in the developing world. Resistance to maize streak virus would be an example. Under this option, Foundation support would focus on identifying and cloning genes that could instill the desired traits. Development of the techniques for gene transfer would be left to others.

The third option was to focus the Foundation's program on a single crop and to develop a fully integrated program ranging from fundamental research through the application of new techniques in breeding, and including socioeconomic evaluation.

Figure 1 and Table 1 indicate the type of assessments made in considering these options. Figure 1 presents estimates of food crop production in the developing world on a dry-weight basis. The cereals account for nearly three-quarters of the production, with rice alone accounting for one-third. Of the noncereals, cassava and sweet potato have the highest Third World production. The first column in Table 1 is based on Figure 1 and indicates relative production levels. The other columns were prepared following a survey on research being conducted on each crop and required a fair amount of subjective judgement.

Figure 1. Developing Country Food Crop Production 1982
(edible dry matter)



Sources:
Edible dry matter is calculated from gross production given in the 1982 FAO Production Yearbook using conversion factors adapted from Wilkes (1982) and Hanson et al (1982). Sugar cane is excluded since it is primarily an export crop.

Table 1. Priorities for Crop Focus of RF Biotechnology Program.

	Importance of Crop in LDCs	Maturity of IARC and LDC Breeding Programs	Sparcity of Biotechnology Research Effort	Probability of Near-Term Success
Rice	****	****	****	**
Cassava	**	**	****	***
Wheat	***	****	**	**
Millet	**	**	****	*
Maize	***	***	*	**
Sorghum	**	***	***	*
Sweet Potato	**	*	***	***
Potato	*	***	*	****

**** = relatively high
* = relatively low

We believe an effective Rockefeller Foundation program could have been developed using any one of the three options considered and/or around any one or more of the crops listed in Table 1. Foundation funds are limited, however, and some focus was necessary. At the Rockefeller Foundation, this type of tough program decision is usually made by the Division Director and it was Alva A. App, then the Director for Agricultural Sciences, who recommended to the Trustees a comprehensive vertically integrated program focused on rice. Looking at Table 1, the rationale for selecting rice can be summarized as follows. In terms of production and consumption, rice is by far the most important crop in the developing world. Moreover, projections indicate that in the 1990s and beyond, in many developing countries growth in demand for rice will move ahead of its supply. Rice breeding programs are mature at the international centers such as IRRI and in several developing countries. There are even some who say these breeding programs are reaching a point of diminishing returns with regard to further improvements that can be made using conventional methods. Rice also turned out to be a highly neglected crop with regard to biotechnology. When we conducted the survey in 1983, we could not find a single research program in the U.S. or Europe studying the molecular genetics of rice. In the West there were only a few modest research efforts on rice tissue culture. Even in Japan there was surprisingly little research on the molecular genetics of rice, although some Japanese universities and corporations have since initiated such programs. The only category where rice did not score high was the one concerning near-term opportunities. We realized that a program focused on rice would require a considerable investment of time, effort, and resources before significant results would be seen in the field. In our opinion, the other factors outweighed this concern. Moreover, there would be the option of working on other crops once the rice program had been established. Within the rice focus, a deliberate effort, described later, was made to balance priorities among traits that would be applicable in poor production environments and traits that would contribute most to increasing production.

Wide Hybridization

As an important component of the international program on rice biotechnology, the Foundation is supporting a significant expansion of the wide hybridization program at IRRI, including the work of Foundation field staff cytogeneticist Lesley Sitch. There are 22 *Oryza* species, only two of which are cultivated. There are six known genomes, A-F, in the genus, including tetraploid species, as well as diploids. As indicated in Table 2, these wild *Oryza* species contain many desirable traits. The objectives of the wide hybridiza-

tion program are to 1) transfer useful traits from wild relatives to cultivated rice, 2) generate new variability for use in rice improvement, 3) transfer wide hybridization technology to national rice improvement programs, and 4) lay the foundations for use of new molecular and cellular techniques at IRRI.

Table 2. Agronomically Important Characteristics Identified Among the Wild *Oryza* Species.

Species	2n	Genome	Characteristics
O. nivara	24	AA	Grassy stunt virus resistance
O. rufipogon	24	AA	Source of cytoplasmic male sterility, tolerance to stagnant flooding
O. glaberrima	24	AA	GLH resistance, early vegetative vigour
O. barthii	24	AA	Bacterial blight resistance
O. longistaminata	24	AA	Floral characteristics for outcrossing
O. punctata	24,48	BB,BBCC	BPH, WBPH, GLH resistance
O. officinalis	24	CC	BPH, WBPH, GLH resistance
O. eichingeri	24	CC	BPH, WBPH, GLH resistance
O. minuta	48	BBCC	BPH, WBPH, GLH, blast and bacterial blight resistance
O. australiensis	24	EE	BPH resistance, drought tolerance
O. brachyantha	24	FF	Rice whorl maggot and stem borer resistance
O. ridleyi	48	—	Rice whorl maggot resistance

Source: Progress report prepared by Lesley Sitch and colleagues, IRRI, March 1988.

The wild species of rice, by definition, do not normally cross with cultivated rice. It is necessary to use a variety of techniques to stimulate pollination, to nurture the otherwise abortion-prone embryo through cell divisions to form a mature F_1 hybrid plant, to overcome sterility problems in the hybrids, and, finally, to produce a breeding line with desired new traits. Progress is being made in all these steps.

Numerous progeny from the second backcross of *O. officinalis* to *O. sativa* show resistance to all known biotypes of brown planthopper, as well as whitebacked planthopper. A gene for resistance to bacterial blight was transferred from *O. longistaminata* to IR24. Hybrids have been made with the wild species *O. latifolia, O. nivara,* and *O. rufipogon* as possible sources of genes that would increase the yield potential of cultivated rice. *O. perennis* and *O. rufipogon* are being studied as possible new sources of cytoplasmic male sterility which could contribute to the spread of hybrid rice and help reduce the potential vulnerability of hybrid rice production which currently has a narrow genetic base of cytoplasmic male sterility.

Postdoctoral scientists from developing countries will participate in wide hybridization research at IRRI and facilitate transfer of the technology to their home countries.

Developing a Knowledge Base and Rice Biotechnology Tools

An obvious early requirement for the Foundation's program was to support research aimed at developing for rice the various molecular and cellular biology techniques, protocols, and materials which constitute the tools of biotechnology research. In other words, it was necessary to get rice biotechnology research up to a level comparable with biotechnology research on maize, tomato, potato, and other crops important in developed countries. Our strategy for this component of the program was to encourage university-based scientists in the developed world who were at the forefront of research on plant molecular and cellular biology to make a significant commitment to research on rice.

This suggestion was not always well received. Many scientists argued that work on model plants was just as important and scientifically much more promising. Others noted that the genetics of rice were so poorly understood that a common nomenclature was still lacking. At some land grant universities, faculty scientists heavily committed to work on other crops were discouraged by administrators from working on rice, a crop that was not even grown in their state. Moreover, these same leading scientists had various other funding options.

Fortunately, through continued discussions, a sufficient number of these scientists were attracted to the idea of working on a crop plant that was a staple food for many of the world's hungry people, and of being part of a mission-oriented research program that would seek to use their research results for the benefit of those people. An initial group of laboratories joined the program in 1985.

In most cases, however, it still took a year, sometimes more, to work out routine procedures, like growing rice plants and rice cell cultures, before real biotechnology research could be undertaken. Significant progress is now being made.

Genetic Maps and Markers. One of the nearer-term uses of molecular biology in plant breeding will be the development of genetic maps and markers based on cloned DNA sequences. While not genetic engineering in the strict sense of the term, use of these tools should enable greater and more efficient exploitation of both the primary gene pool in *Oryza sativa* and the secondary gene pool in other *Oryza* species. Markers will enable breeders to know when introgressions of alien chromatin have occurred and whether the genes for a particular trait are present in a progeny plant even if environmental conditions do not permit their expression. Foundation-supported projects in this area are noted below and listed in Table 3.

Table 3. Rice Genetic Maps and Markers.

RFLP Mapping of Rice and Tagging Important Genes	Cornell University, Steven Tanksley
	IRRI, Gurdev Khush
Analysis of Repeated Sequence DNA	University of Georgia, Gary Kochert
Genome-Specific and Species-Specific Probes	OSTROM Institute, France; Gerard Second
	University of Perpignan, Michel Delseny
DNA Spacer Sequences as Species-Specific Probes	University of Missouri, Lynne McIntyre
	CSIRO Division of Plant Industry, Australia; Rudi Appels
In situ Hybridization and "DNA Fingerprinting"	University of Missouri, Perry Gustafson

At Cornell University, Steven Tanksley and colleagues have recently developed and submitted for publication a saturated genetic map of rice chromosomes based on RFLP markers. An RFLP, or restriction fragment length polymorphism, occurs in a population of rice plants when a distinctive segment of DNA present in all individuals varies slightly in sequence from individual to individual, or breeding line to breeding line. By cutting total plant DNA with restriction enzymes, separating the resulting DNA fragments by length using gel electrophoresis, and then probing the gel with a radioactively labeled cloned segment of DNA complementary in sequence to the DNA at or near the RFLP locus, it is often possible to detect differences from breeding line to breeding line in the length of the fragments containing the RFLP locus. Such differences in fragment length provide an indicator or marker of inheritance of that section of DNA and the genes located on or near it. For breeding purposes, the best markers are those that give different fragment lengths corresponding to the greatest number of different rice lines. In practice, the selection of markers is an empirical process. Many DNA clones are tested as probes and those that detect RFLPs are selected as markers because they work. Further selection and culling is based on the need to fill the gaps on the genetic map.

The Cornell RFLP map of rice now consists of 123 DNA clones obtained from Philippine variety IR36. Based on segregation analysis and use of primary trisomics provided by Gurdev Khush at IRRI, all 123 markers have been assigned to linkage groups and positioned on specific chromosomes. All 12 rice chromosomes are now marked by more than one RFLP marker, and the total number of map units exceeds that of the classical map.

Collaborative research between Cornell and IRRI will continue in order to link the RFLP markers to genes for important qualitative traits such as resistance to certain pests and pathogens. RFLP markers should be particularly useful in following genes for traits that are not routinely expressed or easily scored, and in pyramiding genes for traits such as pest resistance. In theory, any trait that can be scored can be tagged with RFLP markers. This includes quantitative traits, and Drs. Tanksley and Khush will also attempt to use an array of markers to tag the loci of important quantitative traits such as drought tolerance.

It should be noted that the RFLP map also benefits molecular biology. It allows any cloned rice gene to be located at its correct position on rice chromosomes. Furthermore, by providing reference points on the rice genome, RFLP markers may allow the cloning of

important rice genes by putting them within reach of molecular techniques such as chromosome walking.

In this regard, Gary Kochert at the University of Georgia is conducting research which complements and will extend the molecular applications of the RFLP work at Cornell. He is conducting a systematic determination of the types of repeated sequence DNA in rice and will place these sequences on the RFLP map. Together, these studies will provide considerable insight into the molecular level organization of the rice genome.

As noted above, wide hybridization is a painstaking and time-consuming process. One of the more difficult problems is detecting introgressions of alien chromatin. The small size of rice chromosomes makes this particularly difficult, using traditional cytogenetic techniques. This is one of the reasons why wide hybridization for rice improvement had not been significantly exploited. Molecular biology can now help by providing species-specific probes for assessing the extent of introgression of alien chromatin in wide hybridization-derived materials and determining its location on an individual rice chromosome.

With Foundation support, a team of French scientists led by Gerard Second at the OSTROM Institute in Montpellier, and Michel Delseny at the University of Perpignan are developing genome-specific probes within the *Oryza* genus.

Similarly, Lynne McIntyre, now at the University of Missouri, and Rudi Appels at CSIRO in Australia are developing probes for DNA of wild *Oryza* species based on spacer sequences. Spacer sequences are untranscribed segments of repetitive DNA which flank functional genes. The exact function of spacer sequences is unknown, but Appels and McIntyre have shown in *Triticum* that many tend to be species-specific and dispersed throughout the genome. Preliminary evidence suggest that this is also true in *Oryza*.

Perry Gustafson and colleagues at Missouri are seeking to use species-specific DNA probes to visualize alien gene introgressions under scanning electron microscopy. This *in situ* hybridization technique will allow determination of not only the presence of alien genetic material but also its physical location on the rice chromosomes. Gustafson's group will also explore the potential of using "DNA fingerprinting" as a technique for studying the genetic makeup and origin of rice plants.

Steve Tanksley's laboratory at Cornell will serve as a repository and distribution center for all rice probes and markers produced as part of the Foundation's program.

Protoplast Techniques. The genetic manipulation of individual cells is an important aspect of plant biotechnology, but it is of modest value unless whole plants can be regenerated from the cells and protoplasts. As indicated in Table 4, the Foundation is making a significant investment in research on development of rice tissue culture and protoplast techniques. The goal is to attain efficient regeneration of whole plants from protoplasts in order to use protoplasts as a vehicle for various genetic manipulations.

Table 4. Research on Development of Rice Protoplast Techniques.

University of Nottingham	E.C. Cocking
Purdue University	Thomas Hodges
Max Planck Institute	Horst Lörz
University of Tsukuba	Hirofumi Uchimiya
IRRI	Javier Zapata

In 1986, E.C. Cocking's group at the University of Nottingham in England was successful. They obtained efficient regeneration of plants from protoplasts of the *japonica* line Taipei 309. This was one of the first reports of plant regeneration from protoplasts of any cereal. The protocol has been extended to several other *japonica* lines. In March 1987, a course was held at Nottingham which provided hands-on training in the regeneration protocol for 30 scientists from most of the other laboratories in the RF network. Each of the laboratories listed in Table 4, as well as others, has now reproduced and confirmed the Nottingham results. Research continues at Nottingham and includes use of intra- and inter-specific protoplast fusion to transfer genes and produce new genetic combinations including cybrids.

Recently, Tom Hodges' group at Purdue has obtained plant regeneration from protoplasts of *indica* lines IR54 and IR52 and from U.S. variety Calrose 76 which has both *indica* and *japonica* lines in its background. High yields of protoplasts are obtained from suspension cultures. When plated, these protoplasts resynthesize cell walls, divide to form small calluses, and, when transferred to regeneration media, form green embryoid-like structures which grow into small plantlets. The plants still need to mature to test fertility, but they appear to be growing normally.

The focus of Hirofumi Uchimiya's research at the University of Tsukuba is on use of protoplast regeneration as a vehicle for genetic transformation. Like several other laboratories in the program, he has shown that protoplasts will take up DNA and show transient expression of foreign genes. Dr. Uchimiya has further shown that the DNA is incorporated into the genome of these rice cells, passed on to daughter cells, and that genes on the foreign DNA are expressed in the resulting callus. He has been able to regenerate plants from the callus and just recently informed us that he has obtained transgenic rice plants.

Genetic Transformation. The genetic transformation of rice through the introduction of alien or modified genes is one of the principal technological goals of the program. In addition to the protoplast work described above, support is being provided for research on a variety of other techniques, as indicated in Table 5. At the annual meeting of the Foundation's program, held at IRRI this past January, significant progress was reported.

At the University of Ghent, Marc Van Montagu's group has shown that *Agrobacterium* vectors bind to rice cells and that rice induces the "virulence" functions of the Ti plasmid that are required for T-DNA transfer. At the University of Leiden, Rob Schilperoort and colleagues obtained numerous roots at a wound site on rice seedlings when the wound is infected with *Agrobacterium rhizomes*, suggesting that T-DNA transfer has occurred. Both results suggest that *Agrobacterium* should be able to serve as a vector for gene transfer to rice, but this is yet to be confirmed.

At Stanford University, Virginia Walbot's lab has demonstrated the utility of electroporation for introducing DNA into protoplasts, as have several other labs. Using electroporation, she is also introducing DNA and obtaining foreign gene expression in rice mitochondria. Genetic transformation of mitochondria could be highly useful to breeders since it would enable them to move foreign genes in or out of a line by making a simple sexual cross.

At Cornell University, Ray Wu's laboratory is experimenting with both protoplast uptake of DNA and use of a particle gun to deliver DNA to intact cells. Like Dr. Uchimiya, Dr. Wu recently reported that he has obtained transgenic rice via plant regeneration from transformed protoplasts. Thus, it appears that genetic transformation of rice has been demonstrated in at least two labs, but much refinement of the protoplast technique and further development of other techniques will be required before rice genetic engineering becomes a routine process.

Table 5. Research on Techniques for Genetic Transformation of Rice.

Agrobacterium-mediated transformation	University of Ghent, Marc Van Montagu
	University of Leiden, Robert Schilperoort
	Washington State University Gynheung An
Electroporation and other protoplast uptake techniques	Stanford University, Virginia Walbot
	Cornell University, Ray Wu
	Others listed in Table 4.
Electroporation of mitochondria	Stanford University, Virginia Walbot
Particle gun delivery of DNA	Cornell University, Ray Wu
Microinjection of protoplasts, embryoids, and inflorescences	Univ. of California, Davis, Bill Lucas
	Max Planck Institute, Horst Lörz
	University of Leiden, Robert Schilperoort
Liposome fusion	Univ. of California, Davis, Bill Lucas

Cloning and Characterizing Rice Genes. The application of genetic engineering to rice genetic improvement will involve the introduction of novel genes and/or the modification of existing rice genes and genetic systems. To engineer a desired genetic change will require understanding of the genes and gene products responsible for important biochemical and physiological functions. Moreover, the engineered genes will need to be expressed properly, in the right organelle, cell type, and tissue; at the right stage of plant development; and with appropriate response to various environmental stimuli. Identifying genes responsible for important traits and understanding the regulation of their expression in rice is a substantial research task. Listed in Table 6 are relevant research projects currently being supported as part of the Foundation's program.

Table 6. Cloning and Characterizing Rice Genes.

ABA-induced genes Rice phytochrome genes Protochlorophyll reductase genes	Rockefeller University, Nam-Hai Chua
Plasma membrane ATPase genes Protein kinase genes	Salk Institute, Chris Lamb
Genes for carotenoid biosynthesis	Yale University, Alice Cheung
Glutelin genes Prolamine genes ADP glucose pyrophosphorylase genes	Washington State University, Tom Okita
Prolamine genes Glutelin genes	Kyoto Prefectural University, K. Tanaka
Waxy genes	Univ. of Georgia, Susan Wessler
Nitrate reductase genes	Washington State University, Gynheung An
Cold shock genes	Stanford University, Virginia Walbot
Actin genes Alpha-amylase genes	Cornell University, Ray Wu

At Rockefeller University, Nam-Hai Chua and colleagues are studying genes regulated by the plant hormone abscisic acid and by light. The ABA work may lead to a better understanding of mechanisms behind drought tolerance and other traits that appear to be correlated with high levels of ABA synthesis.

At Washington State University, research by Gyn An on nitrate reductase genes and Tom Okita on ADP glucose pyrophosphorylase genes may lead to identification of genetic strategies for improving the efficiency of nitrogen utilization and starch biosynthesis, respectively.

Genes for rice storage proteins are being cloned and characterized by Dr. Okita at Washington State, and by Dr. Tanaka in Kyoto, Japan. The major rice storage proteins, the glutelins, are encoded

by at least three gene subfamilies, and the prolamines are encoded by at least two subfamilies. Current research is directed at determining how these genes are regulated at transcription and translation, and the molecular signals responsible for intracellular sorting and packaging of the proteins. The goal is to enhance the nutritional value of rice by increasing total protein and/or improving the digestibility of storage protein bodies. Up to 30 percent of the storage proteins have been shown to be packaged in indigestible forms in rice. Research led by Alice Cheung at Yale University on genes for carotenoid biosynthesis in rice is also aimed at improving the nutritional value of rice, in this case by making rice grain a new source of pro-vitamin A compounds such as Beta-carotene. The genes for carotenoid synthesis are present in rice, as in all photosynthetic plants, but are expressed in photosynthetic tissue and not in the endosperm. Through genetic engineering, it may be possible to modify the regulation of these genes such that carotenoid biosynthesis occurs in the endosperm of rice, as it does in the case of yellow maize.

One of the likely applications of research on the waxy gene of rice, which is being conducted by Susan Wessler at the University of Georgia, will be the identification of regulatory sequences which provide for high-level expression of genes in rice endosperm. Dr. Wessler is also using the waxy gene to study possible indigenous as well as introduced transposable elements in rice. The identification or introduction of a transposable element would provide a powerful tool for tagging and isolating important rice genes.

Diagnostic Tools and the Study of Host-Pathogen Interaction. Bacterial blight and blast are two important diseases of rice worldwide. Cultivar resistance is often short-lived due to changes in the pathogens. As indicated in Table 7, support is being provided for collaborative research with IRRI conducted by Jan Leach at Kansas State University and Sally Leong at the University of Wisconsin on the pathogens causing bacterial blight and blast, respectively. Near-term goals are to 1) study the genetics of these pathogens and their ability to overcome resistance, 2) identify race-specific genes and develop probes for rapid race diagnosis, and 3) better understand the molecular mechanisms controlling pathogenesis. The long-term goal is to use the knowledge generated to produce more durable resistance in rice.

Table 7. Diagnostic Tools and the Study of Rice-Pathogen Interactions.

Research on *Xanthomonas campestris* pv. *oryzae*, the bacterial blight pathogen	Kansas State University, Jan Leach
	IRRI, Hei Leung
Research on *Magneportha grisea* (*Pyricularia oryzae*), the blast pathogen	University of Wisconsin, Sally Leong
	IRRI, Hei Leung

Novel Genes for Rice Improvement. Genetic engineering should make it possible to incorporate essentially any gene from any source into rice. Identifying alien genes which can instill useful traits in rice is an exciting and expanding aspect of the rice biotechnology program and of plant genetic engineering in general. Relevant research projects currently funded by the Foundation are listed in Table 8.

Table 8. Novel Genes for Rice Improvement.

Viral genes for resistance to rice tungro virus	John Innes Institute, Roger Hull
	Washington University, Roger Beachy
	IRRI, H. Hibino
B.t. toxin genes for resistance to yellow stemborer and other insects	IRRI and collaborators
Maize gene for endosperm carotenoid biosynthesis	Iowa State University, Don Robertson
Wheat genes for inhibitors of rice weevil amylase	Kansas State University, Gerald Reeck
Apomixis in wild *Oryza* species	Univ. of Calif., Davis Neil Rutger
Pennesitum genes for apomixis	Univ. of Georgia, Wayne Hanna

An exciting recent accomplishment in plant genetic engineering was the demonstration by several groups of resistance or tolerance to viral infection in transgenic plants containing viral genes. It should now be possible for many plant virus pathogens to identify candidate viral genes which, when introduced into a plant's genome, can instill this new type of "cross protection." With Foundation support, scientists led by Roger Hull at the John Innes Institute and Roger Beachy at Washington University are collaborating with IRRI to characterize the rice tungro viruses at the molecular level in order to identify candidate genes for resistance to tungro infection. Similar research on other rice viruses will soon be initiated.

It has now also been demonstrated that transgenic plants expressing toxin genes from *Bacillus thuringiensis* insecticides are resistant to insect feeding. There are hundreds of *B.t.* strains, and each toxin is specific to particular insect pests, with the *Lepidoptera* species being the most susceptible. The Entomology Department at IRRI has initiated a research program to screen *B.t.* strains for toxicity to the yellow stem borer and other insect pests of rice. Once toxic strains are identified, they will collaborate with molecular biologists to isolate the genes for transfer to rice.

Two projects are currently being supported aimed at identifying genes from other cereals which may be useful if incorporated into rice. One, led by Gerald Reeck at Kansas State University, seeks to isolate genes from wheat which code for inhibitors of digestive enzymes of both the wheat and rice weevils, whose larvae infest stored grain. The goal is to isolate wheat genes which provide a good source of genetic resistance to weevils and transfer them to rice which lacks a good source of resistance to very similar weevils.

The research program at Iowa State University, led by Don Robertson, is aimed at isolating the Y1 gene from maize using transposon tagging. The Y1 locus of maize is thought to code for a regulatory element which allows carotenoid biosynthesis in the endosperm of yellow maize. The goal is to use the Y1 gene, or knowledge concerning its function, to turn on carotenoid biosynthesis in the endosperm of rice.

The Foundation seeks to identify and support additional research aimed at identifying and isolating other novel genes with potential to instill useful traits in rice.

Setting Priorities and Analysing Impacts

Shortly after the International Program on Rice Biotechnology was initiated in 1985, the Foundation began an in-house assessment of the agronomic traits that should receive priority.

The process of setting priorities consisted of first listing all the potential problems that affect rice which could be addressed using biotechnology. For each problem, estimates of importance were made using several criteria. One consideration was the recognition that the benefits of new production technologies go not only to producers through reductions in the cost of producing rice, but also to consumers through reductions in the price of purchased rice. Only producers who adopt a new technology can benefit from it through lower costs, so the particular type of technological innovation is important. For these reasons, biotechnologies that could solve rice problems using little or no capital investment or purchased inputs and which affected a large mass of Third World rice farmers have higher priority than biotechnologies requiring purchased inputs or which could only be adopted by a few farmers.

Another consideration was the expected probability that a production technology to solve each problem could be successfully developed using biotechnology approaches. Stated another way, this criterion is the estimated length of time it would take to develop a technology to solve each problem. These first two considerations primarily reflect producers' interests.

A third consideration is the quantity of additional rice that would be produced due to a given biotechnology. This primarily reflects consumers' interests. If public policies, such as price supports, which can prevent efficiency gains of technological change from benefiting consumers are not implemented, all consumers gain in proportion to the quantity of rice they consume. From the consumers' viewpoint, the most important aspect of a potential biotechnology is the additional rice it will produce and the resulting price reduction.

Finally, desired traits that have been relatively difficult to introduce using conventional approaches were given higher priority than traits easy to introduce with conventional approaches.

A system of "weights" associated with each problem or technology was provided so that problems affecting the most deserving farmers and technologies with positive environmental impacts could be given greater priority. The weights are, of course, arbitrary, but they can be used to illustrate the effect different configurations of

research priorities are likely to have on consumers and on various categories of producers.

Technologies addressing problems in upland conditions were given "equity weights" of 3, those for deep-water and rain-fed lowland conditions, weights of 2; and those for irrigated conditions, weights of 1. It was assumed that $0.2 million would be invested on each problem annually. A social rate of time preference was used to discount the expected weighted costs and benefits of solving each problem from the year of expected solution to the present.

Problems effectively addressed by conventional approaches were assigned "biotechnology potential" scores of 0.5. Problems for which the likely effectiveness of biotechnology is unknown were given scores of 1. Problems for which conventional approaches have been ineffective even with heavy investments were given scores of 2, as were problems for which there are indications that biotechnology may offer especially effective approaches. An aggregate biotechnology potential score was derived by multiplying the score of conventional approaches by the score for biotechnological approaches.

Table 9 shows four alternative priority listings for the 68 problems included in the analysis ranked according to (1) current value of rice lost to each problem or not produced because of each unmet opportunity for raising potential productivity, (2) current value of output lost, adjusted by equity weights; (3) the equity-weighted value of output lost, discounted to its Net Present Value; (4) the equity-weighted NPV multiplied by the biotechnology potential score for each.

Data in the table are in million dollars, representing increases in productivity that are distributed among classes of producers and consumers in complex patterns. Zero or negative values at the bottom of the first ranking mean that those problems were each estimated to result in increased production valued at less than the discounted NPV of $0.2 million annual research costs. Comparing the ranking between columns gives an idea of the effect that applying the specified weights and biotechnology potential scores has on the rankings.

The results of this analysis indicate that the problems that are widespread and highly yield-limiting in any of the agroecologies and for which there is a reasonable chance that biotechnology can provide a solution should be addressed using biotechnology. Among the insect pests, the highest priorities include gall midge, brown planthopper, yellow stemborer, leaf folder, storage insects,

Table 9. Rank Ordering of Research Problems by Alternative Criteria.

Value of Output Foregone (mil $)		Equity-Weighted Value of Output Foregone (mil $)		Equity-Weighted NPV (mil $)		Equity-Weighted NPV BT Potential (mil $)	
Weeds	1699	Upland drought/blast & iron deficiency	8537	Brown planthopper	1944	Tungro virus	6905
Tungro virus	1534	Tungro virus	7907	Tungro virus	1726	Yellow stemborer	3781
Upland drought/blast & iron deficiency	1423	Weeds	7103	Gall midge	1292	Gall midge	2583
Greater lodging res.	1092	Brown planthopper	5361	Greater lodging res.	1228	CMS	2322
Brown planthopper	1059	Gall midge	4216	CMS	1161	Upland drought/blast & iron deficiency	1962
CMS	1032	CMS	3335	Upland drought/blast & iron deficiency	981	Brown planthopper	1944
Submergence	750	Greater lodging res.	3106	Yellow stemborer	945	Submergence	1685
Gall midge	704	Yellow stemborer	2918	Submergence	842	Greater lodging res.	1228
Birds	601	Submergence	2608	Weeds	718	Seedling vigor	1080
Yellow stemborer	516	Drought at anthesis	2156	Seedling vigor	540	Ragged stunt virus	621
Seedling vigor	481	Birds	2050	Birds	412	Drought at anthesis	575
Drought at anthesis	421	Apomixis	1775	Cold at seedling	310	Waterlogging	524
Apomixis	402	Seedling vigor	1753	Drought at anthesis	288	Leaffolder	400
Cold at seedling	278	Bacterial blight	1261	Apomixis	275	Weeds	359
Bacterial blight	246	Cold at seedling	1018	Bacterial blight	274	Sheath blight	336
Waterlogging	235	Rodents	982	Waterlogging	262	Cold at seedling	310
Coastal saline and acid sulphate	230	Waterlogging	885	Coastal saline and acid sulphate	256	Apomixis	275
Rodents	223	Coastal saline and acid sulphate	783	Sheath blight	168	Coastal saline and acid sulphate	256
Crabs	209	Storage insects	770	Storage insects	158	Birds	206
Sheath blight	152	Sheath blight	767	Ragged stunt virus	155	Storage insects	158
Storage insects	143	Ragged stunt virus	703	Rodents	151	Bacterial blight	137
Ragged stunt virus	141	Crabs	682	Crabs	141	Blast	135
Leaffolder	92	Hispa	508	Striped stemborer	132	Striped stemborer	132
Hispa	88	Grain discoloration	495	Whitebacked hopper	121	Whitebacked hopper	121

Table 9. Rank Ordering of Research Problems by Alternative Criteria. (cont.)

Value of Output Foregone (mil $)		Equity-Weighted Value of Output Foregone (mil $)		Equity-Weighted NPV (mil $)		Equity-Weighted NPV BT Potential (mil $)	
Grain discoloration	80	Leaffolder	479	Leaffolder	100	Grain discoloration	104
Striped stemborer	73	Striped stemborer	416	Blast	68	Rodents	75
Whitebacked hopper	67	Blast	392	Alkaline soils	63	Drought at seedling	75
Blast	63	Whitebacked hopper	338	Hispa	57	Crabs	71
Drought at seedling	60	Drought at seedling	308	Grain discoloration	52	Alkaline soils	63
Alkaline soils	59	Alkaline soils	221	Drought at seedling	38	Hispa	57
Sheath rot	28	Mealy bug	152	Sheath rot	28	Hoja blanca	29
Mealy bug	26	Sheath rot	138	Grassy stunt virus	26	Sheath rot	28
Grassy stunt virus	26	Grassy stunt virus	129	Leaf scald	21	Grassy stunt virus	26
Leaf scald	21	Grain-sucking bugs	123	Iron deficiency	16	Leaf scald	21
Grain-sucking bugs	21	Leaf scald	112	Mealy bug	15	Grain-sucking bugs	21
Iron deficiency	17	Iron deficiency	83	Iron/mang toxicity	11	Armyworm	17
Iron/mang deficiency	13	Thrips	68	Grain-sucking bugs	10	Iron deficiency	16
Thrips	12	Armyworm	66	Thrips	10	Mealy bug	15
Armyworm	11	Hoja blanca	60	Green leafhopper	9	Iron/mang toxicity	11
Hoja blanca	9	Iron/mang toxicity	51	Armyworm	9	Thrips	10
Green leafhopper	6	Udbatta	34	Hoja blanca	7	Whorl maggot	9
Ufra	6	Ufra	34	Udbatta	3	Green leafhopper	9
Udbatta	6	Green leafhopper	31	Ufra	3	Ufra	3
Ants	4	Ants	27	Whorl maggot	2	Udbatta	3
Aluminum toxicity	3	Diopsis	17	Diopsis	0	Aluminum toxicity	0
Diopsis	3	Aluminum toxicity	12	Aluminum toxicity	0	Diopsis	0
Whorl maggot	2	Whorl maggot	12	Ants	-1	Ants	-1
Caseworm	1	Root nematode	5	Grain processing	-2	Early maturity	-2
Yellow mottle virus	1	Yellow mottle virus	5	Grain quality	-2	Grain quality	-2

Table 9. Rank Ordering of Research Problems by Alternative Criteria. (cont.)

Value of Output Foregone (mil $)		Equity-Weighted Value of Output Foregone (mil $)		Equity-Weighted NPV (mil $)		Equity-Weighted NPV BT Potential (mil $)	
Root nematode	1	Caseworm	5	Caseworm	−2	Grain processing	−2
Black bug	0	Black bug	3	Yellow mottle virus	−2	Black bug	−3
Mole cricket	0	Mole cricket	1	Black bug	−3	Brown spot	−3
High temperature	0	High temperature	0	Seedling maggot	−3	Cold at anthesis	−3
Grain processing	0	Grain processing	0	Peat soils	−3	Vitamin A	−3
Grasshopper	0	Grasshopper	0	Leaf streak	−3	White grubs	−3
Acid sulphate soils	0	Acid soils	0	Cold at anthesis	−3	Leaf streak	−3
White grubs	0	White grubs	0	Vitamin A	−3	Acid soils	−3
Vitamin A	0	Vitamin A	0	Brown spot	−3	Peat soils	−3
Peat soils	0	Peat soils	0	White grubs	−3	Root nematode	−3
Acid soils	0	Seedling maggot	0	Acid soils	−3	Mole cricket	−4
Brown spot	0	Root aphid	0	Early maturity	−3	Root aphid	−4
Naranga	0	Naranga	0	Root nematode	−3	High temperature	−4
Root aphid	0	Acid sulphate soils	0	Mole cricket	−4	Naranga	−4
Seedling maggot	0	Early maturity	0	High temperature	−4	Acid sulphate soils	−4
Cold at anthesis	0	Cold at anthesis	0	Grasshopper	−4	Grasshopper	−4
Grain quality	0	Grain quality	0	Acid sulphate soils	−4	Seedling maggot	−6
Leaf streak	0	Brown spot	0	Naranga	−4	Caseworm	−8
Early maturity	0	Leaf streak	0	Root aphid	−4	Yellow mottle virus	−9

striped stemborer and rice hispa. Among the diseases, the highest priorities include tungro virus, ragged stunt virus, blast (if at the same time a greater degree of upland drought tolerance can be introduced), sheath blight, and bacterial blight. Among physical environmental conditions, the highest priorities are submergence tolerance to flash floods, tolerance to waterlogged conditions or chronic stagnant water of 1-3 feet depth, upland drought tolerance, lowland drought at anthesis and cold temperature at the seedling stage. Among the opportunities for raising potential productivity are greater lodging resistance, cytoplasmic male sterility, greater seedling vigor, and apomixis. Additional problems that are highly ranked despite the lack of any apparent genetically determined means of control are weeds and birds. Both are especially important in Africa, as well as in other areas where upland rice is important.

The results of this analysis are being used as a general guide for allocating the Rockefeller Foundation's resources addressed to identifying genes for rice improvement (Table 10), but the allocation decisions are tempered by the recognition of the following weaknesses in the analysis:

Table 10. Rank Order of Priority Traits for the Rockefeller Foundation's Rice Biotechnology Program.

Resistance to tungro virus
Resistance to yellow stemborer
Resistance to gall midge
Cytoplasmic male sterility
Drought tolerance
Resistance to brown planthopper
Submergence tolerance
Greater lodging resistance
Seedling vigor
Resistance to ragged stunt virus
Tolerance to drought at anthesis
Tolerance to waterlogging
Resistance to leaffolder
Resistance to sheath blight
Cold tolerance at seedling
Apomixis
Tolerance of coastal saline/acid sulfate conditions
Resistance to bird damage
Resistance to storage pests
Resistance to bacterial blight
Resistance to blast
Resistance to striped stemborer
Resistance to whitebacked planthopper

—The estimates of area and yield lost to each problem for Latin America, the Middle East, and North Africa are purely nominal; estimates of experts familiar with those areas must be obtained, as well as better data for the other regions.

—The estimates of success with conventional approaches and the potential for biotechnological solutions need further improvement, and, in fact, the latter will change as researchers discover new ways to use the evolving tools.

—The use of arbitrary weights to reflect environmental and equity considerations leaves much to be desired. At the least, additional experiments on the model to discover the effect of alternative sets of weights will be conducted.

—Alternative approaches to incorporating the equity and environmental dimensions will be sought, as well as practical alternative ways to reflect the fact of diminishing returns to research on individual problems.

It is not always easy to predict the impact of technological change, as illustrated by the "unintended consequences" that have been identified with the Green Revolution. A part of the rice biotechnology project is directed at generating a more complete understanding of the consequences of technical change. This includes a series of research projects in China, India, Indonesia, Bangladesh, the Philippines, Thailand, and Nepal (Table 11) that compare areas where semidwarf rice varieties and fertilizer have been widely adopted with areas in similar socioeconomic conditions where the new technology has not spread widely. Emphasis is on the differential impact of the technical change on the factors of production including careful examination of differences in labor migration, wage rate changes, land rental and sales, and capital and input use.

Preliminary analysis indicates that in a number of countries labor has migrated from nonadopting to adopting areas, and agricultural wage rates have increased in the same proportion in both adopting and nonadopting areas. Because similar analysis is being conducted in a number of countries, it should be possible to determine the interaction of technological change with socioeconomic conditions. Researchers will be challenged to consider what agronomic traits might be incorporated in rice to have the optimal socioeconomic impact.

Table 11. Setting Priorities and Determining Impact.

Future supply and demand for rice	International Food Policy Research Institute, Howarth Bouis
Priority traits for rice biotechnology	Rockefeller Foundation, Robert W. Herdt
Technological change in Nepal's hill agriculture	Cornell University and IRRI, Krishna Belbase
Differential impact of new rice technology in Thailand	University of Khon Kaen and Kasetsart University, Sarun Wattanutchariya
Differential impact of new rice technology in India	Indian Statistical Institute, Sudhin K. Mukhopadhyay
Differential impact of new rice technology in Bangladesh	Bangladesh Rice Research Institute, Bangladesh Institute of Development Studies; Mahabub Hossain
Differential impact of new rice technology in Indonesia	Center for Agro-Economic Research, Faisal Kasryno
	Gadjah Mada University, Tumari Jatileksono
Differential impact of new rice technology in China	Rural Development Research Center Justin Yifu Lin
Methodology for the study of the differential impact of new rice technology	IRRI, Cristina David, Keijiro Otsuka, and Yujiro Hayami

Building Capacity in Developing Countries

Initially, a major portion of the Foundation funding committed to the rice biotechnology program was used to support research in advanced laboratories to assure development of the knowledge base and technologies required for the genetic engineering of rice. Progress has been made in gaining that knowledge, and a greater portion of the funds will now be used to facilitate transfer and use of new rice improvement technologies in the developing world and to help build the scientific capacity for further development of rice biotechnology in selected developing countries. This includes support for research and a major commitment to training.

A substantial effort has been and continues to be made by the International Rice Research Institute in the Philippines to develop and maintain a productive network of rice researchers throughout the world. IRRI scientists constitute a key central component of the Foundation's rice biotechnology program by collaborating in both applied and basic research. The work of IRRI helps to assure that research supported by the Foundation is relevant to priority needs of developing countries, and that the results and benefits of the research reach those countries. CIAT plays a similar role in Latin America and IITA in Africa.

Since 1985, the Foundation has been providing support to rice breeding programs at the institutions listed in Table 12 for research on the use and improvement of biotechnologies such as anther culture that can be applied today. With completion of the RFLP map, an important new research technology will soon be available, and plans are currently underway for appropriate workshops and facilities to permit its transfer to the developing world. We are also in the process of identifying several more national rice research institutions which will be invited to join the program.

Table 12. Use of New Techniques for Rice Genetic Improvement.

China National Rice Research Institute, Hangzhou
Korean Rural Development Administration, Research Bureau
Shanghai Academy of Agricultural Sciences, China
International Rice Research Institute, Philippines
International Center for Tropical Agriculture (CIAT), Colombia

Last year, the Foundation initiated discussions with officials in China and India concerning opportunities for a significant expansion of Foundation support for rice biotechnology research in those countries. It will include research and training on the knowledge-generating and technology development aspects of the program, as well as applications in rice breeding. We believe that Chinese and Indian scientists will make important contributions to all aspects of the program, and we anticipate that the resulting national rice biotechnology programs will, over time, assume more and more responsibility for further development and refinement of rice biotechnologies.

Arrangements for implementing this new phase of the Foundation's program are proceeding most rapidly in China. The Foundation has signed a Memorandum of Understanding with the China National Center for Biotechnology Development (CNCBD) of the State Science and Technology Commission for a cooperative program entitled "Biotechnology Development for Rice Improvement." CNCBD, which has national responsibility for structuring, promoting, and supporting biotechnology initiatives in China, does not itself conduct research but rather works through existing Chinese research institutions to accomplish its goals. Last year, a call for proposals from Chinese scientists with competitive evaluation by international professional peer review led to the selection of 23 research proposals at 15 institutions as the initial group participating in the Chinese component of the program. CNCBD and the Foundation share research funding and oversight responsibilities. Participating institutions are eligible to nominate their best young scientists for Rockefeller Foundation predoctoral, postdoctoral, and career development fellowships.

In India, officials of the Department of Biotechnology in the Ministry of Science and Technology and of the Indian Council of Agricultural Research have expressed enthusiasm for Foundation assistance in establishing a similar coordinated national program on rice biotechnology. Discussions are currently underway concerning the details.

Biotechnology and Donor Opportunities: Experiences in India

Kerri-Ann Jones
Biotechnology Advisor
USAID/New Delhi, India

It is generally accepted that biotechnology holds especially great potential in the developing world. Having acknowledged this fact, how do those interested in development begin to support this complicated and continually evolving area? Initially, it is important to develop a list, matching technical advances with development problems. Once the list is established, the next step is to identify priorities. What topics should be targeted? Thanks to numerous meetings and worldwide discussions, this phase is of identification and priority setting is well underway.

It is now time to take a broader view of biotechnology as it becomes entrenched and its potential begins to be realized. Products are beginning to move into the marketplace. This article attempts to provide an overview of donor opportunities by looking at biotechnology in its entirety. It suggests the kinds of programs that are needed to support the application of biotechnology to development problems. The discussion includes descriptions of experiences in India, where many programs are being put into action.

It is hoped that the reader's conclusion drawn from this article will not be one of frustration with an overly complicated subject but rather one of excitement at the possibilities and challenges ahead.

The Big Picture

In order to discuss the opportunities for donors in biotechnology, it is necessary to have a clear and complete view of this complex area. As it encompasses the entire spectrum from basic research to marketing, it presents a multitude of possibilities. In presenting such numerous possibilities in varying areas, biotechnology is a new kind of crosscutting theme for development experts to consider.

Biotechnology can be viewed as the intersection of a number of scientific disciplines. The recent rapid advances are the results of incremental discoveries in biology, chemistry and genetics. The transformation of these discoveries into useful technologies is dependent on production engineering. Finally, the use of a new product in society requires the interaction of scientists, research administrators, government officials and entrepreneurs.

In a development context, biotechnology can also be described as the intersection of multiple approaches and efforts. Some of these are traditional elements of development programs, such as institution building and training. These are fundamental components of many development projects and are essential in biotechnology, which is based on technical skills. Efforts in these areas build on long-term support to improve a country's research capacity; for example, in agriculture or health sciences.

Biotechnology also encompasses other approaches to development, such as stimulation of private enterprise and trade and investment concerns. These recently rediscovered elements of development strategies will clearly be affected by growth in biotechnology. The establishment of a biotechnology industry will add another facet to the private sector, as well as stimulate already existing industries. Biotechnology also presents excellent opportunities for trade and investment. New products for worldwide export and joint venture possibilities are numerous.

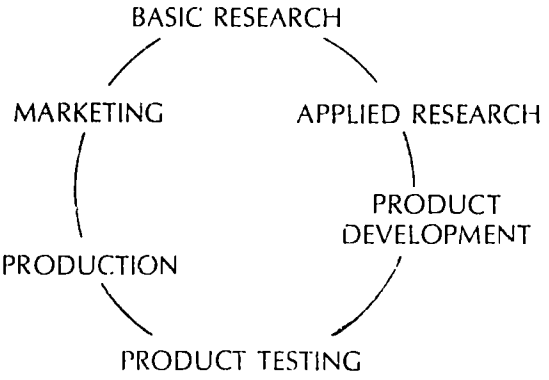
In order to be useful, biotechnology must move from the laboratory into the field. This is especially true in development programs, which seek to improve the well-being of the largest number of people. The mechanismsfor movement vary. Products may be distributed through government programs or commercialization. The method is basically unimportant, as long as it works and the results are finally put to use.

A simplistic description of the biotechnology pathway is:

RESEARCH → PRODUCTION.

This is, of course, a most naive view and it has been responsible for much of the overselling which has plagued biotechnology. The actual steps through the cycle in biotechnology are similar to those in any research and development endeavor (described in Figure 1). A problem is attacked at the basic research level, moves to applied research, then through development, testing and production. The cycle is complete when the product can be used in the field by the general public. This process is ongoing. It continues as the feedback from product use reveals needs and leads to refinements.

Figure 1.



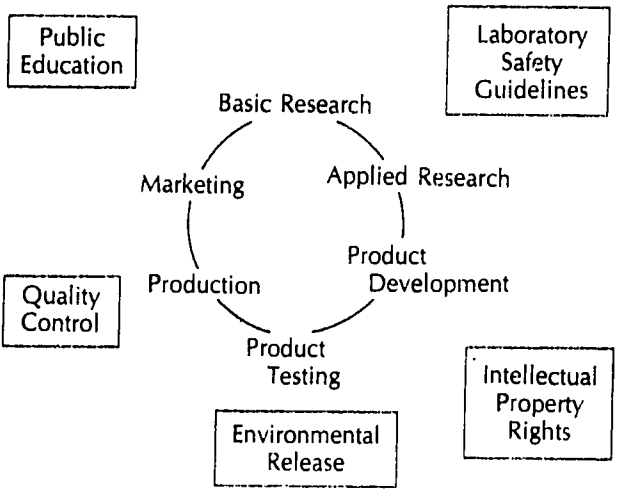
The cycle can be divided into two general categories: those concerned with research and those concerned with utilization. Although the steps can be easily defined, none of them can be viewed as a certainty or problem-free. Each requires specific skills and time. In the early days of biotechnology, the time factor was often underestimated. Many of the necessary skills require a mixture of old and new technologies; for example, plant breeding and cell biology, or fermentation technology and molecular biology.

In addition to the basic steps outlined above, biotechnology is surrounded by a constellation of issues that are as important for movement as any technical skill. Figure 2 depicts the more complete view of biotechnology.

The concerns and requirements surrounding biotechnology include laboratory safety guidelines, intellectual property rights, environmental releases, quality control and public education. These have arisen as a result of the new methods and types of products that are becoming available, the most significant method being recombinant DNA technology.

As the initial steps in biotechnology have taken place at the laboratory bench, it was necessary to determine the safety of the new techniques. Consideration of safety in biotechnology began with the Asilomar Conference in 1975, which led in the U.S. to the NIH Recombinant DNA Advisory Committee and the NIH Guidelines. As more experience has accumulated, these Guidelines have been revised and they have been accepted as worldwide models and standards for laboratory procedures.

Figure 2.



As the commercial potential of biotechnology became glaringly obvious, the question of ownership became an issue. Who owned the ideas and the new products? What was patentable? Environmental questions arose as products needed to be tested in the field. Quality control procedures for new kinds of products, such as monoclonal antibodies, needed to be developed.

Regulations are being developed worldwide, and discussions in international forums are increasing, including working groups in OECD, NATO and UNIDO/UNEP/WHO. Most countries are wrestling with these issues, interpreting old procedures and standards and establishing new ones. For example, in the U.S. the first genetically engineered animal has recently been patented. However, the possibility exists that there will be a moratorium on future patents of this kind, as many groups feel that this policy has not been adequately reviewed.[1]

The importance of public education in the use of biotechnology has become increasingly apparent. The private sector has learned the value of discussing product testing at the community level, as local opposition to proposed testing has resulted in delays. As governments explore and define their levels of support for the new technologies, they are seeking to educate and determine public opinion. One such effort is a background paper on "Public Perceptions of Biotechnology" prepared by the Office of Technology Assessment of the U.S. Congress.[2]

India has a solid and expanding scientific infrastructure, with one of the world's largest scientific communities. By 1990, it is anticipated that India's scientific community will number 3.1 million. In 1987, India devoted 1% of its GNP to research and development and related science and technology activities.[3] However, despite these impressive statistics, India remains confronted by many basic development problems. In 1984-85, over 35% (36.9%) of the nation's people were living below the poverty line, defined by India's Planning Commission as unable to provide themselves with the minimum requirement of 2400 calories per day.[4]

The Government of India recognized early the importance of biotechnology for both continued scientific growth and their attack on developmental problems. In 1982, within the Department of Science and Technology, a National Biotechnology Board was established. In 1986, this Board was expanded and elevated to the level of a separate department within the Science and Technology Ministry. The Department of Biotechnology has identified six areas of activity. These are: manpower development, infrastructure facilities, research and development programs, technology missions, science and technology missions, and international collaboration.[5] The identification of these areas parallel activities in most countries as they approach biotechnology. First, adequate facilities and skilled individuals must be established and given ongoing support. Following this, projects of importance to the country are identified. In India, one of the Government's technology missions is to increase production of edible oil to overcome current severe shortages, which have forced India to rely heavily on imports. The Department of Biotechnology is active in this mission, supporting research in plant tissue culture for the micropropagation of high-yielding types of coconut oil and palm oil.

The largest item in the DBT budget is biotechnology research. Included under this item are training, infrastructure facilities, projects and R&D units for product generation. The last activity indicates that the Government of India realizes the necessity of supporting the entire spectrum of biotechnology. For example, DBT is supporting the development of the National Biochemical Engineering Research Center/Pilot Plant. The purpose of this institute is to "bridge the gap between laboratory findings and an industrially viable fermentation process."[6] Also funded under this category is the National Biotechnology Information System, a national communication network.

Donor Opportunities

Most of the development programs that incorporate biotechnology have grown out of the recognition of the importance of science and technology. Biological research programs that were keeping up-to-date with new methods found biotechnology becoming part of the project. In this way, the newer methods were naturally being incorporated into ongoing programs. Therefore, most donor support has focused on the research portion of the biotechnology cycle, either by supporting developed country scientists in their efforts to address specific development problems or by supporting increased research capacity in developing countries through training and infrastructure development. This two-pronged approach should culminate in the establishment of truly collaborative research programs.

Biotechnology research possesses some unique qualities. It is extremely interdisciplinary in nature; it is based on rapidly evolving technologies; and it involves significant participation by industry. These qualities present donors with new opportunities, which are essential for success.

The interdisciplinary nature of biotechnology is responsible for its existence and its ultimate success. Advances in numerous disciplines led to the development of new technologies. And, in order for any advance to move out of the laboratory, basic and applied researchers must work together. An important activity for donors is the support of creative programs that ensure that all the necessary players are involved. For example, a plant tissue culture program will not progress very far without the participation of a plant breeder.

One of the most exciting elements of biotechnology is the continuous appearance of new methods and technologies. Such a rapidly moving field requires constant training and retraining. Focused training programs to learn specific techniques are essential. Also, continual revolving training is needed to allow a scientist to keep up-to-date and build on his/her repertoire of skills. Donors can play an important role in developing such targeted courses or providing access for developing country scientists to already existing programs.

The industrial involvement in biotechnology is not only critical in commercialization efforts but it is also the site of basic research. Industrial laboratories are carrying out a large amount of basic research and development, in both highly focused programs and others, more academic in nature. Any program in biotechnology must recognize the importance of industrial activities. One activity,

which would be of great value, is an industrial postdoctoral program, where scientists from developing countries could train in private sector companies in the developed country. Not only would such training provide technical advancement, but also the specific nature of industrial biotechnology research could be observed. In addition, the support of some model activities linking the public and private sectors would be valuable. In the developing world, the gulf between academia and business is still quite large.

Utilization of biotechnology products, the second half of the cycle, presents completely new challenges. Both the private and public sector are novices in this area. Established pharmaceutical companies are going through a learning process because of the new types of products that are becoming available, and governments are straining to develop balanced policies that ensure both safety and growth.

Commercialization of biotechnology is finally moving in the private sector. More products are coming into the market, and the number of products in field testing is increasing. In the developing world, progress is slower but interest in the private sector is beginning to grow. In India, large companies such as Hindustan Lever, Tata and Southern Petrochemical Industries Corporation (SPIC) are developing biotechnology programs. Additionally, Indian industry is becoming aware of the need for linkages to academic institutions, and grants from companies to university scientists are appearing. More and more medium- and smaller-size Indian companies are requesting information on biotechnology and are attempting to identify U.S. companies from whom they can obtain technical assistance.

In India, USAID and the GOI have a program known as Program for Advancement of Commercial Technology (PACT), which is providing some useful experience in the commercial area. The purpose of the project is to stimulate industrial R&D through Indo-American joint ventures, leading to the commercialization of innovative technical products and processes to the benefit of both economies. Although not designed specifically for biotechnology ventures, there are an increasing number of proposals in the area.

PACT works by supplying financial assistance to Indo-U.S. joint ventures. The program provides half of the product development cost (up to $500,000). The support is a conditional grant, repayable on predefined terms if the product is successful. The program is administered by the Industrial Credit and Investment Corporation of India (ICICI), which awards the individual grants. ICICI is a development finance institution established in 1955 to encourage and assist industrial development in India.

The majority of biotechnology proposals submitted to PACT have been in the biomedical area, specifically for diagnostics. More and more proposals are appearing in agriculture, however, with suggested products including modified strains of *Bacillus thuringiensis*, modified fungal strains for the production of enzymes, seedlings for reforestation, and vaccines for poultry diseases. All of these are joint proposals between Indian and U.S. companies.

All of these proposals have certain problems in common. Often they are unfocused and would require longer than the 2-3 year time frame which PACT requires for bringing products to the market. It has become obvious that Indian and U.S. companies have much to learn about each other. Based on this experience, USAID has supported activities to improve the situation. A biotechnology workshop was held for Indian and U.S. companies last year, and USAID is currently working on the preparation of a technical report on the U.S. biotechnology industry. This report will serve as a handbook for Indian companies to facilitate their interaction with the U.S. corporate community.

The policy questions that confront the developing world are no different from those facing the developed world. The two central issues are regulation and patenting. Regulations for the use of biotechnology products are evolving worldwide, and several developing countries are using developed country regulations as models. In India, the guidelines on "Recombinant DNA Research Safety in India" are now being revised and expanded to move beyond laboratory and basic research Considerations include: "(i) laboratory research; (ii) manufacturing industries; and (iii) application (environment and practice) in areas of recombinant microorganism, plant rDNA, embryo transfer, gene therapy, vaccines, etc."[7] The Indian system has borrowed heavily from the U.S. regulatory system, most notably adopting the Institutional Biosafety Committee and defining varying classes of risk.

Two important aspects of regulation in the developing world must be considered. First, there are unique environmental factors in the tropics, which comprise a large part of the developing world. In the area of plant genetic engineering, a recent workshop of U.S. experts concluded that, in general, the risks involved in the field use of engineered plants is minimal. This judgement is limited to the U.S., however, where few wild relatives of food crops exist.[8] In the developing world, the location of so many of the centers of origin, the release of engineered plants will be more complex. Second, implementation and monitoring of the regulations is complex and difficult in the developing countries. This has not been a simple task for the developed nations, which have much more experience in

regulatory and environmental issues. In developing countries, the existence of adequate infrastructure and personnel necessary to support all of the essential reviews and monitoring procedures is highly questionable.

India's patent system, based on the Patent Law of 1970 and further elaborated in the Technology Policy Statement of 1983, is quite different from that of the United States. India does not allow product patents in a number of areas, including agriculture and health, the leading areas for application of biotechnology. Furthermore, in these areas, process patents are only allowed for specific substances. The existence of weak patent systems or none at all is common in the developing world. Collaboration in commercial biotechnology between the developed and developing world may be seriously limited by these different views of patent protection.

The donors' role in these policy areas is one of facilitation. Developing countries need to be involved in the ongoing international discussions, balancing the experiences of the developed world with their own needs and cultures. A constant sharing of information is a necessity for developing countries to become aware and active. In conclusion, the possibilities for donors in biotechnology are numerous. They require much thought, creativity and flexibility. This technology is new to everyone. In many ways, a worldwide learning process is occurring and Donors' aid should ensure that developing nations are full participants in this process.

References

1. William Booth, *Science* (Washington, D.C.) 240,718 (1988).

2. U.S. Congress, Office of Technology Assessment, New Developments in Biotechnology—Background Paper: Public Perceptions of Biotechnology, OTA-BP-BA-45 (Washington, D.C., U.S. Government Printing Office, May 1987).

3. *Research and Development Statistics 1984-1985,* Government of India, Department of Science and Technology, New Delhi.

4. *Seventh Five-Year Plan 1985-1990, Mid-Term Appraisal,* Government of India, Planning Commission.

5. *Annual Report 1986-1987,* Government of India, Department of Biotechnology, Ministry of Science and Technology.

6. *Ibid.*

7. *Ibid.*

8. *Regulatory Considerations: Genetically Engineered Plants*, San Francisco, California: Center for Science Information, 1988.

Development of Guidelines and Regulations for Safe Conduct of Agricultural Research

Sue Ann Tolin
Department of Plant Pathology, Physiology and Weed Science
Virginia Polytechnic Institute and State University
and U.S. Department of Agriculture

It is indeed a pleasure to address this international group on considerations of the biological safety of agricultural biotechnology research. After many of the comments in and during discussion of talks during the last three days, I feel it is particularly important that this topic was included as a part of this symposium.

I should state from the outset that within USDA, I am associated with Science and Education and will be speaking today primarily from my experiences with the research activities of the Department. I will discuss regulations briefly, but my expertise is not in that area and I do not consider myself a "regulator" of biotechnology. Instead, I am a concerned university scientist conducting research with plant viruses, and spending a portion of my time with USDA on this issue. I have had the unique opportunity to observe the federal government's role as a facilitator as well as a regulator of research and development, and have been involved in countless meetings on the issue of agricultural and environmental applications or releases of genetically engineered organisms.

My remarks are directed primarily to the safety of biotechnology involving molecular gene transfer by recombinant DNA methods. This is done for several reasons—first, recombinant DNA is the method that first raised concerns among the scientific community and still raises public concern. And second, guidelines for safe conduct of research were initially directed toward recombinant DNA technology since it enables specific gene transfer between widely different organisms, and is perceived by some to be a less certain and higher risk method than conventional methods of genetic manipulation. Although I will not dwell on the differences, definitions need to be clear because of the perception that all genetic engineering modifications are done by recombinant DNA methods, and thus are biotechnology and need strict regulation.

My emphasis on recombinant DNA technology does not mean that I differ with the excellent recent report of a National Academy of Science committee, chaired by Arthur Kelman, which concluded that "there is no evidence of unique hazards either in the use of R-DNA techniques or in the movement of genes between unrelated

organisms," that "risk associated with introduction poses no risks different from the introduction of unmodified organisms and organisms modified by other methods," and that "assessment of risk should be based on the organism, not the method of engineering."

Biotechnology in the broad sense has always been utilized in agriculture with tremendous benefits accruing from genetic modification of organisms. In the narrow sense, biotechnology is only beginning to be utilized in agriculture, and after the presentations at this meeting, I need say no more on its potential. Questions of its biosafety, and of its subsequent overregulation, however, may preclude that this powerful tool is ever used for the benefit of agriculture, either domestic and international. The questions that must be addressed are: Does biotechnology pose any greater environmental risk than classical agriculture? Is it perhaps even safer?

The application of biotechnology to medical problems is widely accepted today, and products are on the market. Why is it that progress in biomedical biotechnology was made so rapidly and public acceptance was gained? It is my contention that the promulgation of the Guidelines for Research Involving Recombinant DNA Molecules was the turning point that allowed research to proceed within microbiological laboratories throughout the country and indeed throughout the world, since these guidelines quickly were accepted as the international standard. And, as you recall, the Guidelines were promulgated as an answer to broad safety and ethical concerns raised initially by the scientific community. The concerns became sufficiently great in the public sector to cause proposed legislative and executive actions at federal, state and local levels in the mid-70s. There was, importantly, no new legislation and no regulation through application of existing public health or worker safety statutes.

The National Institutes of Health was named as the lead federal agency for promulgation of these guidelines. The NIH established the Recombinant DNA Advisory Committee, or RAC, to advise it on actions to take in regard to approving experiments and in changing the Guidelines as scientific results became available regarding the risk posed by the research. The public process of review of the Guidelines and review of specific experiments, together with the recognized expertise of the members of RAC seemed to alleviate concerns of the public toward recombinant DNA research—at least for nearly a decade, until proposals came to utilize organisms modified by recombinant DNA technology for research in experiments conducted not in a laboratory, but in a

field. I speak, of course, of the ice minus bacteria experiments in a California potato field.

Development of Guidelines—A Historical Prospective

I think it is useful now, to go back a few years, and review actually how these guidelines were developed, and that the concept of guidelines was at that time a unique approach to the resolution of a "sticky" question. Then, as today, the issue was just as much political and social as it was scientific.

Concerns that there may be risks associated with biotechnology were first expressed over a decade ago when gene splicing techniques were discovered. Scientists were unsure of what the consequences might be of moving genes, in particular of the possible effects on humans working in the laboratory with genetically-engineered *E. coli*. This bacterium was, incidentally, widely used as a research tool, and was thoroughly understood genetically.

The first risk scenario concerned the transfer of genes from SV40, a virus that was associated with cancerous tumors in certain animals, into *E. coli*. The questions that were asked then were: What would happen if some of that *E. coli* got into the gut of a laboratory scientist? Would it multiply? What would happen to that foreign viral DNA as the bacterium multiplied? Would the genes on that DNA be expressed in her body and cause cancer? What would happen if the recombinant bacterium passed through her gut and ended up in sewage treatment plants? Would it survive there? Would the foreign DNA be transferred to other bacteria? What would its fate be if it were transferred?

Scientists at that time did not know the answers to these questions and rightfully expressed concern to their colleagues and to the public. A moratorium on research with recombinant DNA molecules was called for and various legislation was proposed in Congress to require strict regulation of this research because of the conjectured risks.

The concern over the safety of recombinant DNA research was discussed at a number of workshops and scientific meetings. At one of the first workshops held now over a decade ago, the Asilomar Conference, a group of eminent scientists was asked to develop a set of guidelines for conducting research with recombinant DNA molecules to allow certain experiments to proceed safely by complying with these guidelines. The Guidelines called for conducting research under certain specified containment conditions, both *physical* and *biological*, and prohibited the conduct of certain

experiments because they were, at that time, judged to have too uncertain an outcome. Part of today's situation regarding the biosafety of release experiments dates back to those early days of uncertainty when the technology was new and decisions were very conservative, and deliberate release into the environment of an organism containing recombinant DNA was included as a "prohibited experiment."

Under the Guidelines, *physical containment* meant that elaborate laboratory structures, facilities and operational procedures were to be utilized to restrict the number of bacteria accidentally escaping during normal laboratory operations, and to prevent contamination or infection of laboratory workers. These were based largely on standard laboratory practices codified by the Centers for Disease Control for research in microbiological and biomedical laboratories. If either the organism or the isolated gene posed a recognizable high risk, such as being associated with a serious disease of man or animal, the most stringent physical containment conditions were called for. Non- or weakly pathogenic organisms and genes required much less stringent physical containment because of recognizably lower risks. Again, the CDC had already classified organisms based on level of risk, and the RAC used their classification.

In the Guidelines, *biological containment* was introduced specifically to reduce the risk of recombinant DNA-containing organisms that might be accidentally released into the environment or accidentally carried out by workers in the laboratory. It called for using *E. coli* strains that could not infect humans, did not survive and spread outside the laboratory, and could not transfer the introduced foreign genes readily into other organisms that were in the environment.

As I said earlier, the National Institutes of Health was named as the lead federal agency for promulgation of these guidelines. The NIH established the RAC to advise it on actions to take in regard to approving experiments and in changing the Guidelines as scientific results became available regarding the risk posed by the research. The NIH Guidelines became the federal government guidelines, since all other federal agencies, including the Department of Agriculture, were asked in 1976 by executive order to require that these same guidelines be followed for research they funded. Each other agency had a nonvoting representative to the RAC. I have played that role for USDA since 1979. The Congress concluded that the RAC/Guideline process was adequate to protect the public from the potential or perceived risks of recombinant DNA research,

and no new legislation was enacted. The moratorium was lifted and research proceeded.

The first version of the Guidelines was so restrictive that initially only scientists in medical laboratories with high containment facilities could conduct recombinant DNA research. Cloning human DNA was prohibited in the early days of the Guidelines, as was cloning toxin genes. There was even a ban on shotgun cloning, on experiments to increase the pathogenicity to plants of plant pathogens, and on culturing greater than 10 liters of a recombinant DNA-containing organism. Deliberate release of an organism containing recombinant DNA into the environment was also on the prohibited list, as mentioned earlier.

Upon the advice of the RAC, the NIH gradually allowed additional research based on increased knowledge of the organisms and the techniques. The RAC at first approved all recombinant DNA experiments, then as experience was gained, designated physical and biological containment levels needed for certain ones, and passed all review on to the local level by an Institutional Biosafety Committee. The RAC also designated certain experiments as exempt from the Guidelines because they posed no risk greater than that of working with the nonengineered organism. There were several classes of research that were deemed of low risk and thus could be done, because the resulting organisms were no different than organisms that could and do form by natural genetic processes. Finally, organisms other than *E. coli* were included as "safe" organisms.

The progress made in the last decade in biomedical and health care sciences has been tremendous, not only in results of research, but also in the facilitation of the research by the decisions of the RAC and NIH. The RAC, in fact, is prepared now to receive proposals for conducting human gene therapy experiments. But the RAC and NIH's role in nonmedical research has been mixed.

Approvals have been granted by the NIH to release DNA-modified plants and microorganisms in controlled field tests. The long experiences with plants and animals and their associated microorganisms in agriculture certainly had demonstrated that field experimentation with organisms altered by traditional genetic engineering methods could be conducted safely. The RAC, following their customary procedures for moving categories of experiments from case-by-case consideration by the RAC to generic cases that could be reviewed by the IBC, added Appendix L to the Guidelines to describe a category of rDNA-containing plants that should be of lower concern in experiments conducted according to conditions described in the

Appendix. The RAC recommended that under Appendix L, such experiments were to be considered by the Plant Working Group rather than the entire RAC. Parallel appendices, M, N, and O for microorganisms, animals and vaccines were envisioned at a meeting of the RAC last year, but have not yet been acted on.

Although the requests to conduct plant and bacterial experiments in the field were approved because no untoward effects to humans or the environment could be envisioned, the legality of this action was challenged because NIH did not prepare an Environmental Impact Statement in accordance with the National Environmental Policy Act, and because it had not developed a procedure stating how it will address all issues dealing with releasing genetically engineered organisms. Currently, this is the key issue in the application of biotechnology to the benefit of agriculture. Agriculture is in a "Catch-22" situation because risks cannot be assessed without release, and release under the Guidelines was not permissible without assessing all of the potential risks.

Additional complications arose in the willingness and authority of the NIH to give approvals for environmental releases, since NIH is not a regulatory agency. The Guidelines had, however, become *de facto* regulations for the biotechnology community, both public and private. The regulatory agencies, including EPA and USDA/MIS, claimed jurisdiction even at the research level if a field test were involved. Debates within the federal government and in numerous workshops and forums have occurred during the last 4-5 years.

It is recognized widely that research with genetically modified organisms is ready to be conducted outside the confines of a microbiological laboratory in conditions more appropriate for the growth of the organisms, such as a whole plant or animal. However, the NIH Guidelines give no guidance to investigators and to IBCs for designing containment facilities to conduct this research safely, not even in growth chambers or greenhouses, or in barns or feedlots. USDA/S&E recognized this void and drafted initial proposals to the RAC to assure the biosafety of research conducted with organisms utilizing these facilities. These proposed additions to the Guidelines describe containment conditions to provide for the safe conduct of research with plants, animals and organisms associated with them that contain recombinant DNA molecules. They were reviewed and modified by RAC Working Groups, then were approved by the RAC at the September 1987 meeting. The NIH is currently considering these additions, and a new version of the Guidelines incorporating them is expected soon.

The proposed additions do two things that parallel what was done for biomedical research covered by the Guidelines. First, accepted scientific practices for handling these organisms safely is codified; and second, criteria for establishing levels of risk of microorganisms that are pathogenic to plants are established by which assignment to physical containment levels is made.

Current Status of Regulation of Biotechnology

The Office of Science and Technology Policy published an extensive document, the Coordinated Framework for Regulation of Biotechnology, in the Federal Register (26 June 1986) in which the applicability of various statutes of U.S. regulatory agencies was reviewed. It states essentially that "the existing regulatory framework is adequate to regulate biotechnology." I will in no way try to describe the entire Coordinated Framework, but will state briefly the areas of agency jurisdiction.

The Coordinated Framework included a Final Rule from EPA describing their jurisdiction over microorganisms under FIFRA and TSCA. USDA/MIS/APHIS in the Framework and later on 16 July 1987, published a Final Rule regarding regulation of plants based on the Plant Pest Act. This Final Rule of APHIS is now in effect and is coordinated by the Biotechnology and Environment Coordinating Staff (BECS). It has been used by several industrial biotechnology companies to obtain permission to grow recombinant DNA modified plants in field tests, and the results of some of those field tests were presented earlier in this meeting. The Framework also describes USDA regulation of veterinary biologic products under the Virus-Serum-Toxin Act. The position of the Food and Drug Administration over their traditional areas in pharmaceuticals and human health care is described. FDA also reserves authority to look at foods that may come from biotechnology-derived plants and animals.

Congress is again examining the situation and proposing legislation. Various groups are examining the authorities of the above acts for regulation of biotechnology. Time will tell if the fruits of biotechnology will be applicable to agriculture, or whether the risks, real or conjectural, and the accompanying regulations will prevent rapid utilization of this technology for agriculture. Congress is also concerned about maintaining the U.S. competitive position in biotechnology.

Guidelines Versus Regulations

The Department of Agriculture has been a strong proponent of the NIH Guidelines and envisions the need for guidelines for agricultural biotechnology research that closely parallel the NIH Guidelines both in content and impact on the discipline. The USDA Guidelines, an advanced notice of which was included in the Coordinated Framework in 1986, were "envisioned to encompass all phases of agricultural biotechnology research" including that research conducted in specialized isolation research facilities as greenhouses and barns, and environmental research releases in controlled and segregated field plots and animal pens. The rationale for the Guidelines is the concept that research in biotechnology, conducted under an appropriate set of research guidelines, will not result in products for commerce that require regulation different from products in which biotechnology was not used in their development. This is essentially the same conclusion reached in the National Academy of Science report.

Accepting this rationale reduces potential risks perceived to be associated with the new biological organisms to a common form or class based on generic properties that are defined, selected for, and monitored during the research process. Defining what these properties are is a task that could be undertaken by the RAC or by the Agricultural Biotechnology Research Advisory Committee (ABRAC), recently formed by USDA. Establishing these generic properties, which is essentially a form of risk assessment, should contribute to a more orderly and scientifically based regulation of products where that is needed. It should be noted that "where needed" suggests that regulation of all products is not necessarily needed. Many should fall in the category equivalent to GRAS, or Generally Recognized as Safe.

The second rationale is the recognition that conditions for safe conduct of research beyond the containment provided by laboratories can be incorporated into guidelines, rather than designing case-by-case conditions for each experiment such as described by Monsanto in their tomato field trial. The descriptions of conditions could encompass the entire range from growth chamber, phytotron and glasshouse, to field containments involving climatic, edaphic, genetic, biological, chemical and other forms of containment designed for special uses. The entire research process would be maintained under the guideline structure for oversight. The USDA's ABRAC intends to discuss further development of such guidelines as one of their initial actions.

The USDA also envisions a value in establishing a National Biological Impact Assessment Program (NBIAP) as an important feature in the total strategy for the development of biotechnology. The rationale for this program is the concept that utilization of biotechnology-derived agricultural organisms—plant, animal, microorganisms—will require assessment of impact over time from a biological basis as well as from a social and economic standpoint. Since it may be envisioned that large masses of biota will be distributed through many ecosystems, the impact might be measured only by a stepwise process over time. An assessment system of this magnitude will be essential to verify predicted performance and to monitor effects systematically. It will prove equally important in integrating biotechnology into production systems in agriculture. Thus, major concerns that have been raised by the possibility of changing climatic and edaphic factors, mass population effects, spontaneous genetic events, intrusion of exotic organisms, and other factors can be more readily anticipated and adjustments provided. The efforts of NBIAP may allow the efforts to protect our agricultural systems by classical concepts of exclusionary biology, or quarantines, to be reduced in emphasis.

Current and Future Problems

The process of developing guidelines and reviewing specific experiments in a public forum, together with the recognized expertise of the members of RAC at the national level and the IBC at the local level, seemed to alleviate concerns of the public toward laboratory recombinant DNA research and eventually allowed complete exemption from the Guidelines of most recombinant DNA research. There was even once the proposal that the Guidelines be reduced to a Code of Standard Practices for Recombinant DNA Research. The question I raise is whether we can follow this model of history and do the same for agricultural biotechnology research.

Resolution of this question may ultimately influence whether we are going to be able to use biotechnology in agriculture. If the answer is no, why not? Is it because it will not work? Is it because we can't test it to see if it works? Is it because we can't afford to test it, because of the cost of obtaining data required by regulatory agencies to obtain approval for a test to see if it works? Or is it because it becomes simpler for the scientist, public or private, to *not* do certain experiments? Is it because public or private funds for support of the underlying basic science decrease in anticipation of the regulatory hurdles? Or will issues of proprietary rights, patents, and confidential business information discourage investment in agricultural biotechnology?

And if the answer is yes and biotechnology is used in agriculture, can we do it safely and wisely? How will we know if biotechnology is used safely? It has been said that biotechnology is okay to be used in biomedical research, but not in agricultural or environmental applications. Critics also say that biotechnology in agriculture will increase the use of chemicals, principally of herbicides because plants are being made to be resistant. But others say that biotechnology has the potential to decrease the use of chemicals drastically. We have certainly heard that prediction here at this meeting.

Many statements have been made to the effect that if a solution is not found in a timely manner to the special constraints currently placed on conducting this research, it would delay or prevent the development of the full potential of biotechnology for this nation and affect our competitiveness. An even worse outcome is the prospect that investigators would proceed without proper concern for the consequences and in so doing encourage a climate of reckless development of biotechnology in agriculture, natural resources and the environment. Another outcome, already discussed here, is to move to international settings for testing—to countries without regulations.

The National Academy of Science report essentially says there are no problems —develop guidelines and proceed. But as I stated earlier, guidelines for physical and biological containment of research outside of traditional laboratories with plants and animals and associated microorganisms are not yet incorporated by NIH into the Guidelines and leave containment conditions to the discretion of the IBC. Regulatory agencies have been reluctant to say that generic guidelines for release experiments can be developed, and at this point in time are conducting a case-by-case review of each release request.

Industry in the United States is saying essentially that they now have a mechanism through the Coordinated Framework which establishes the statutory authorities for the regulatory agencies to approve field trials, but are recognizing the high cost of the approvals. Will the high cost confine requesters to large private industry? Will the regulatory decisions be right? And, will the fact that the tests are regulated make them safe?

Future Needs and Recommendations

I believe that agricultural and environmental applications of genetically-engineered organisms should be addressed in a

scientifically-sound, openly-debated, decision-making process. It should include public policy and involve scientists, industry, and all interested parties much like the RAC did. Conditions should be established to let research with small scale, and limited extended field trials proceed with minimum of regulation. Experiments should be stratified based on risk, rather than putting everything into high risk categories because of the process by which it was developed. Finally, regulation should be at the product level, not on the research which is needed to develop the product. Research must be done in an open and free manner, at least in the public sector, to see if products have potential to impact agriculture, either in a positive or negative manner.

Scientists need to do a much better educational job than we have in the past. I think this should be followed by an assessment of the impact a particular release has had on biological systems, particularly in agroecosystems which occupy a large percentage of this country.

Regulation should be based on demonstrated risks, not on those that are conjectural, and it should be distributed among the agencies based on what the agency has traditionally covered for functions like product licensing, registration, and certification. It should be based on the application and use of a new product and not on the process by which it was developed. The cost of data collection involved in obtaining permission to conduct a test should also be based on what the risk is, not what can possibly be measured. Research exemptions should be allowed for determining whether a biological application will be effective, and following product licensing, continued observations of its impact should be made.

Collaborative efforts of scientists throughout the world are still needed in the area of biotechnology. I see that these efforts could be directed toward the following areas:

1) Establishing criteria for safe practices and safe organisms;
2) Codifying risks for each crop, geographic area, agroecosystem;
3) Codifying standard, scientifically accepted practices for conducting agricultural research that could be considered as good developmental practices worldwide;
4) Addressing alternatives to the use of exclusionary biology, quarantine, and physical containment for assuring safe conduct of research; and
5) Developing monitoring systems and data bases to tie in with national and international agricultural networks.

International Agricultural Research and Biotechnology: Strategies for Safety

Anne K. Hollander, Associate
The Conservation Foundation
Washington, DC

Introduction

The conceivable benefits of biotechnology for the developing world are impressive in both quantitative and qualitative terms. Increased food production, better disease control, and new sources of renewable energy are among the potential gains which, if realized, could help to solve many of the most intractable problems of the less developed world. The desire to realize these gains has infused the international agricultural research community with a justifiable sense of excitement and hope.

In the midst of the inspiring discussions of the benefits of biotechnology for the developing world, it is important also to acknowledge that some risks will attend these benefits. The advantage of acknowledging these risks is that it allows us to consider how we may reduce or avoid them. Three risks in particular seem to deserve our attention: (1) the risk of failing to accomplish the most important benefits of biotechnology, (2) the risk of unexpected environmental harm, and (3) the risk that economic changes which accompany the new technologies will occur at such a pace or on such a scale that the developing countries cannot adequately manage them.

This paper is organized as follows. The first two sections describe the potential benefits of biotechnology for developing countries, and the world agricultural setting into which biotechnology is being introduced. I then describe three significant risks that could hinder the gains we otherwise anticipate from genetic engineering research. In conclusion, I suggest a strategy—one which should be viewed as experimental and flexible— which people and institutions who work in the international agricultural research community could use as a starting point to develop their own ways of addressing these risks.

Benefits of Biotechnology Research

By some estimates, approximately 50% of agricultural yield increases achieved since the turn of the century are due to improved genetic characteristics.[1] This is a strong indication of the potential

of biotechnology, which it opens previously unimaginable avenues for improvement of genetic traits. Among the projected benefits and goals of research in crop breeding are better understanding of genetic and physiologic processes in plants, faster diagnosis of plant diseases, improved resistance to stresses (salinity, pests, diseases, drought, and herbicides) and the ability to propagate species that are difficult to propagate vegetatively. There may be significant improvements in food quality due to increases in nutritional content and more genetic variability in plant varieties. Faster screening for desirable traits and faster breeding cycles can reduce the time from research and development to actual use. As one example discussed at this conference, the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) has overcome fertility barriers in peanuts using genetic engineering. This has allowed them to cross the commonly cultivated varieties with a dozen wild relatives that have better resistance to late leaf spot and rust.[2]

Beyond the agricultural sector, other important potential benefits of biotechology include savings of scarce and expensive energy resources, new approaches to conservation of genetic resources, and new pharmaceuticals to treat human and animal diseases. These benefits, if realized, may translate into second-order benefits for the countries that adopt them such as increased export earnings, expanded employment opportunities, and geopolitical advantages due to increased national income.

Historical Perspective on International Agricultural Research

Genetic engineering is being introduced into a global agricultural setting which differs in important ways from the setting into which agricultural innovations were introduced in the past. Reviewing these differences sheds light on the question of whether biotechnology's effects will differ markedly from the effects of past technological innovations.

The characteristics of the global agricultural setting that have changed most in the last 25 years can be summarized as follows:

(1) Changes in agriculture in one part of the world now have direct implications for agriculture in other parts of the world;

(2) Society views science and technology as potentially dangerous and yet strategically important; and

(3) The direction of agricultural research and development is now primarily determined by the private sector rather than the public sector.

Interdependence of Agricultural Economies

Until relatively recently, the agricultural practices and policies of individual countries often did not have major ramifications extending beyond their own borders.[3] Now, agricultural policies and practices are globally interdependent. For example, when agricultural innovations occur, they create jobs for people in one part of the world, but often at the expense of jobs in another part. When trade barriers are erected, they protect the agricultural sector of one country at the expense of others. Furthermore, agricultural development is influenced by the policies of international development assistance agencies.

G. Edward Schuh noted the significance of this phenomenon for society:

> "Our new place in an international economy raises important moral and ethical questions, for consequences of our actions now go far beyond our own body politic. . .[International developments] expand the context in which ethical and distributional issues arise and complicate the consideration of such issues because of the different sets of values brought into play when considering the international scene."[4]

Attitudes Towards Science and Technology

A second thing that has changed in the last quarter century is society's view of science and technology; in particular, its view of the relationship of science and technology to economic development and strategic matters.

In the early 1960s, most observers believed that science and technology were "a positive force for development"; that science would "reduce the gap" between rich and poor countries. Since that time, the general public and scholars alike have questioned this concept.[5] Science and technology has even been blamed by some for being the *cause* of many problems in developing countries, including environmental contamination, overly rapid rates of urbanization, and the unequal division of labor between the industrialized countries and the developing countries.[6]

Little evidence suggests that science and technology are the sole or predominant cause of many developing country problems. However, we have come to realize that technological developments have very different impacts depending on the social, economic, cultural, and environmental systems into which they are introduced.[7]

Our view of science and technology has also changed in that today we regard it as a strategic matter that enhances national power. Political leaders subsidize research and development (R&D) much more extensively than in the past, and we see more emphasis on preventing access to the results of R&D especially if they are considered strategically important. Strategic R&D includes not only that which has military relevance, but that which has economic importance.[8]

Control of Agricultural Research Has Shifted

The third major change in international agriculture in the last 25 years is that the driving force behind agricultural research has changed. Basic research used to be conducted primarily in the laboratories of public universities; today private companies play the dominant role. In addition, both vertical and horizontal integration is accelerating in the industries involved in this research. In other words, the companies that dominate agricultural R&D also control the other four components of the world food production system: production of inputs, agricultural production itself, processing of foods, and international distribution of foods.[9]

The three new characteristics of the global agricultural setting—interdependence, strategic importance, and private control—affect the likelihood that the three risks discussed in the next sections may come to pass.

Will Society Realize the Most Important Public Benefits of Biotechnology?

Many of the potential benefits of biotechnology will be achieved; we already see significant progress on new human and animal drugs, and new crop varieties. However, some of the most important products that this technology could produce may not be available for a long time, if ever. A few examples include waste degradation techniques, low-toxicity pesticides, pesticides that are targeted to specific pests and therefore have limited environmental impacts on beneficial insects, and vaccines against the major endemic diseases of the developing countries. From an international development perspective, important potential benefits also include improved employment opportunities for the poorest populations, and improved human health.

Unfortunately, some obstacles may significantly delay the realization of these benefits. The four most significant obstacles are the following:

(1) Technical constraints,

(2) Lack of economic incentives for companies to develop some of the most important products,

(3) Inadequate infrastructure to disseminate the products to those who need them most, and

(4) Insufficient public sector research.

This section describes each of these four potential obstacles.

The technical problems of achieving goals in the laboratory and of applying those findings to large-scale production are well documented.[10] Scientists are still in the earliest stage of unlocking the secrets of heredity. They have barely begun to understand the physiology of plants and their ecological relationship to other organisms—for example, over 90% of all soil microorganisms cannot yet be cultured in the laboratory, much less identified or studied.[11] Therefore, technical and scientific constraints are certainly a formidable problem in realizing biotechnology's potential. Yet we are overcoming these constraints with a speed that consistently outstrips previous estimates. Furthermore, unlike the other three constraints I will discuss below, technical problems on the whole tend to be "socially neutral." In other words, scientific gaps inhibit all applications of biotechnology equally; they do not by themselves create a bias in regards to the social uses to which the technology is put.

A second obstacle to achieving potential benefits is that the private sector has insufficient economic incentive to develop many socially useful applications of biotechnology. As one example, consider narrow-spectrum pesticides. An important advantage of genetic engineering is that it permits the development of pesticides targeted to only one pest and one crop at a time. Such pesticides are particularly beneficial because they are effective yet cause limited adverse effects, e.g., they do not kill insect predators or cause human illness. However, such pesticides tend to be poor business investments for at least two reasons. For one, the potential market for a single-pest, single-crop pesticide is usually very small, especially compared to the market for a multipest, multicrop pesticide. Second, the fact that the public derives benefits from the narrow-spectrum pesticide because of its safer environmental characteristics has little or no economic relevance to producers. The marketplace tends to impose the same financial and time costs for research, development, regulatory approval, and marketing on every pesticide product, regardless of differences in environmental superiority.[12]

As another example, human vaccines are critically needed—especially to reduce the endemic diseases of tropical countries—but liability risks and the inability of end users to pay for the product has a strong dampening effect on development of such products. When the president of Genentech (a U.S. biotechnology firm) was asked by the World Health Organization to participate in developing a vaccine for malaria, he declined. The potential economic gains were insufficient to justify the cost and the financial risk.[13]

A third obstacle to realizing important benefits of biotechnology arises because of the lack of infrastructure to test and disseminate products in poorer countries. For benefits to be realized by end users, applied research must be integrated with an infrastructure for marketing, manufacturing and distributing final products. In developing countries these factors are in chronically short supply. There is a shortage of experienced researchers, research managers and senior executives; of production and marketing infrastructure; of scale-up facilities; and of the risk capital needed to finance new businesses.[14]

The case is frequently made that the private sector is best equipped to enhance the dissemination and application of new technologies; the public sector should conduct research and develop products that otherwise would not be developed, while the private sector distributes them. This concept is encouraging public-private collaboration within the U.S. and in the international research community.[15] If research directions are not influenced by this collaboration, it may be a good solution to the infrastructure problem. However, the inherent orientation of the private sector is to make profits. Thus, it may be difficult, in collaborative ventures, to prevent this orientation from causing public needs research to be diverted away from public needs, towards applications that have higher potential profitability, e.g., cash crops instead of subsistence crops.[16,17]

In conclusion, there appear to be arguments for the International Agricultural Research Centers, the Agency for International Development, and the rest of the publicly funded agricultural research community to concentrate—perhaps exclusively—on research priorities for which there is not sufficient financial incentive for private investment. Otherwise, society may lose an important weapon in the effort to achieve the benefits of biotechnology: namely, a strong and independent public research sector.

Environmental Risks

Most but not all organisms developed for release to the environment are expected to present low risks to the environment, especially at the small field-test stage.[18] Still, most experts agree that environmental uses of genetically engineered organisms should be assessed for risks on a case-by-case basis, because engineered plants, animals, and microorganisms will present very different risks depending on such factors as their motility, reproductive capabilities, and potential pathogenicity or toxicity.[19] As mentioned earlier, these risks must be considered in light of the expected benefits. However, using engineered organisms on a broad scale will present some challenging environmental issues which we should begin to consider now.

Types and Models of Risk

Whether one believes that risks from genetically engineered organisms will or will not materialize depends largely on the ecological models one accepts as relevant. Three different models are usually cited: the laboratory experience model, the classical breeding model, and the exotic species model.[20]

The laboratory experience model suggests that fifteen years of experience in using genetically engineered microorganisms in laboratories has caused no harm, even though we know that some of these organisms regularly escape to the environment.[21] Therefore, we should not expect harm to occur when we deliberately introduce such organisms into the environment. Most ecologists do not accept this model because organisms used in laboratories are dependent on nutrients and temperature controls which are provided for them in the laboratory environment. Thus, they are not expected to survive when they do escape and the fact that "no harm has occurred" is attributable to factors that will not pertain to organisms deliberately introduced to the environment and designed to survive under normal environmental conditions. A second argument that undermines the laboratory experience model is that there is good evidence demonstrating a long lag period before the harmful effects of many substances become apparent. History has demonstrated in the cases of chemicals, cars, and many other inventions that fifteen years is an insufficient period of time from which to draw decisive conclusions about environmental hazards.

The second model sometimes used to predict risks of genetically engineered organisms is the classical breeding model. Its proponents state that centuries of experience with crop breeding have

yielded no serious environmental problems, and that genetic engineering is a logical extension of this experience. This model can also be faulted because crop breeding has not been benign in terms of its environmental impacts. It *has* been tremendously beneficial, and most people believe that on balance the benefits have outweighed the problems. Still, environmental disruption is associated with most forms of modern agriculture including crop breeding, and perhaps its adverse effects could be reduced if they were more widely recognized and if the economic consequences were understood.

The following examples support this conclusion. First, breeding of genetically identical plants, which is encouraged by most plant breeding activities and is becoming even more prevalent with gene cloning, has increased the vulnerability of crops to disease and pests, with some historically serious consequences for management of stable food supplies. The 1970 corn blight in the United States is but one example. It cost farmers about $1 billion in losses and some Southern states lost more than half their crop; it also seriously threatened the 1971 corn seed supply.[22] Another environmental and economic problem with plant breeding as practiced in its modern form is that cultivars bred to produce superior yields or disease resistance sometimes displace land races which contain other types of important germplasm. Such erosion of biodiversity has recently been recognized as a major environmental problem because many factors in addition to agricultural practices are now hastening its spread.[23] As a third example, chemical inputs such as pesticides—on which many genetically improved varieties depend—often kill wildlife and present dangers to humans. Again, the economic costs of such problems are both direct and indirect, but include increased costs for health care, reduced income from wildlife industries, lost revenues when export crops are rejected by importing countries because of pesticide residues, and many other problems.[24] Finally, water salinization, soil erosion, and ecological deterioration from resettlement have resulted in some countries from efforts to expand irrigation to meet the needs of intensive agricultural practices.[25]

From these examples we know that genetic improvement of crops, while enormously beneficial, is not an environmentally neutral activity. It therefore does not provide sufficient basis for concluding that genetically engineered organisms used in agriculture will be environmentally benign.

A third model often cited as relevant to predicting possible risks from genetic engineering is the "exotic species" model.[26] This model suggests that genetically engineered organisms are com-

parable to organisms that evolved naturally in one environment and were subsequently moved to another environment ("exotic" organisms). Experience with exotics may be pertinent in some cases because genetically engineered organisms have *combinations* of traits that are new to the environments in which they are introduced, even though many of their traits will also be found in that environment.

Experience with exotic species shows that such organisms can have highly undesirable effects, that natural communities are invasible, and that introduced species that have characteristics giving them an ecological advantage can spread.[27] One of the most well-known examples of this is the peacock bass which was introduced in the Panama Canal Zone, where it caused the extinction of several native species of fish, and also resulted in an increase in the mosquito population and an attendant increase in malaria in the region.[28] On the other hand, the exotic species model does not hold well in cases where small genetic modifications (e.g., single gene changes) are made to indigenous organisms. Exotic species, at least for now, tend to be more genetically "foreign" to their environments than genetically engineered organisms. As genetic engineering advances, however, we will be able to produce organisms that span the continuum from indigenous to exotic. The exotic species model may be useful for some of these.

In conclusion, no single model from past experience is sufficient to predict the risks of genetically engineered organisms in general, nor do existing models establish that genetically engineered organisms will never present significant risks. Instead, as most experts have concluded in the last few years, risks must be assessed on a case-by-case basis.

Reducing Risks

An interesting and useful characteristic of the tools of genetic engineering is that the same techniques which allow scientists to construct organisms that are economically useful also give scientists the power to reduce potential risks associated with the organisms they create.

To begin with, genetic engineering permits the transfer of single, well-identified genes. Therefore, the nature of resulting organisms can be more predictable than with products of conventional plant breeding or undirected mutagenesis. Scientists can use well-identified and well-studied genes and host organisms to enhance this predictive capability.

Genetic engineering technology also enables scientists to engineer into organisms traits that reduce their rate of proliferation or survival. Some scientists have worked to develop "suicide genes": traits that will cause an organism to perform its intended function and then self-destruct.[29] Such systems are not yet perfected, but they offer an opportunity for scientists to use engineered organisms while minimizing their long-term presence in the environment. Economic incentives tend to encourage the production of organisms (or seeds) that will not reproduce because such organisms can be sold year after year to consumers.

Genetic engineering also permits organisms and even specific traits to be marked and monitored in the environment. The U.S. Environmental Protection Agency's Office of Research and Development has been conducting tests on marker systems since 1984.[30] Monsanto Corporation and Clemson University recently conducted an experiment in the U.S. with an organism which carried no new traits except a genetic marker; the purpose was to test the efficacy of the marker.[31] The advantage of such systems is that they can allow scientists (and regulators) to track organisms and may help determine if they are causing unintended harm. If so, genetic markers can help find organisms in the environment and hopefully mitigate risks if they do occur. Some people have proposed that such marker systems also could play a role in assigning liability if damage occurs.[32]

Incentives to Reduce Risks

Given that some applications of genetic engineering may present risks and that the techniques of molecular biology can be used to reduce some of these risks, we are still left with the question: *are* there incentives for scientists to reduce risks before they occur?

The answer to this question varies among different countries and among institutions within countries. In most scientific endeavors, pressure to realize profits, to publish important findings, and to build the national economy creates an incentive for companies, governments, and laboratories to move ahead quickly with research. These same forces probably encourage them to overlook measures they might take to minimize possible harm. One biotechnologist frankly acknowledged this pressure when he observed that "imagining the profits [from genetic engineering] can unhinge even a sane scientist."[33]

Another disincentive to incorporating safety measures in engineered products is that markets do not reflect the true long-term values of most environmental resources, including land, air, water and

biodiversity.[34] Therefore, the potential profit from organisms which have marker genes and suicide traits, which have minimal chance of genetic transfer, and which involve only well-identified genetic material, is certainly no greater than the same organism without these safeguards—and it costs more to develop the safer one.

Regulations can help to reduce this problem, but adequate regulatory procedures for genetically engineered products are not yet in place in most developing countries and are hard to enforce even where they exist because of the incentives noted earlier.

Although environmental risks of products are assessed, in some countries, it will be difficult and often inappropriate to apply those assessments to other countries. A number of U.S. biologists recently gathered to discuss risks from genetically engineered plants. One of the risks they discussed was the possibility that stress tolerance genes might unintentionally be transferred in the environment to nearby weedy species, making them more difficult to eradicate. The group concluded that only a handful of U.S. crops cross-pollinate with weedy relatives, because most of their weedy relatives are in other (tropical) countries. However, the group strongly emphasized that unintentional gene transfer could be a more serious risk in countries where the wild and weedy cousins of major crops reside.[35]

In closing this assessment of environmental risks, it is necessary to mention the role of public understanding. Public trust in any new technology can be seriously shaken by a single unexpected, adverse event or association.[36] Public mistrust of food irradiation is an example of this phenomenon—it springs from the public's association of irradiation with nuclear weapons and nuclear power plants, which have little relation to food irradiation technology. Similarly, developing countries' reactions to environmental releases proposed in their countries are likely to be strongly influenced by factors other than environmental risk. These factors include the country's view of other agricultural innovations such as Green Revolution technologies, and its public's fundamental trust or distrust of the research and assistance institutions conducting the experiments. Experiences in the U.S. (California, Wisconsin) and in several developing countries (Argentina, India) have already shown that scientists and research institutions will need to consider these factors at least as much as the ecological risks when they propose to conduct environmental release experiments.

In conclusion, the argument that genetically engineered organisms present *no* environmental risks is not persuasive, regardless of the model used. Further, there are incentives for scientists to ignore the

risks. At the same time, the risks are probably manageable if they are acknowledged, and the risks in many cases may be outweighed by the potential benefits. For the public and the governments of developing countries to accept the technology, however, some institution(s) will need to take responsibility for both risk assessment and risk communication. This is the subject of the final part of this paper. First, however, the next section of this paper explores a third and final concern: the possibility of economic disruption.

Managing the Rate and Scale of Economic Change

> "New technologies are never deployed in a vacuum; rather they are introduced within existing environmental, economic, social, and political systems. They cause changes in those systems and they usually benefit one group at the expense of another. If these biotechnologies are to fulfill their great promise, then these impacts and inequities must be anticipated and addressed in the policy system."—Martha Gilliland[37]

Economic risks, as I use the term here, are risks that threaten to undermine the long-term economic opportunities of a country. I want to clearly distinguish such risks from the vast majority of economic changes that are short-term and occur constantly in any society, and which are clearly desirable from the standpoint of economic progress.

Experts in economic development processes who have studied the potential effects of biotechnology have argued that the changes it will bring may cause a variety of economic problems, from reducing developing countries' control over their genetic resources, to crop displacement and lost export markets. In fact, everyone who studies biotechnology acknowledges that eventually it will bring vast economic changes. The important policy question is whether or not there will be time for developing countries to adjust. Clearly, the answer to that question will remain elusive until more research and experience can be gained, but several factors suggest that such questions deserve further attention.

The Gap Between Rich and Poor

Development economists have long debated the question of how economic development can best be ensured; in particular, whether gains to wealthy sectors "trickle down" to the poorer classes, or whether development only occurs through gains made by the poorer classes directly.[38] Regardless of their views on this, both camps agree that economic development can only occur in the long term if the gap between rich and poor people and nations

lessens rather than widens. Therefore, a legitimate concern of development economics is whether biotechnology will increase or decrease these gaps.

What evidence do we have that the gaps may increase? Is the evidence sufficiently strong to cause us to take these risks seriously? At least three reasons suggest we should.

The first reason is because of the "refraction effect." Political scientists have coined this term to describe the common phenomenon that benefits of technological change, as they are passed through the an existing social order, are distributed in rough proportion to the existing distribution of assets and power. This phenomenon has been observed by a number of historians and has occurred throughout history.[39]

A second piece of evidence comes from experience with the Green Revolution, which provides an imperfect though useful historical analogy. Parallels between the Green Revolution and the biotechnology revolution have been drawn by a number of authors.[40] All reach the same conclusion: biotechnology has tremendous potential for benefit, but may also cause negative economic and social effects similar to those seen after the Green Revolution—except biotechnology's effects will be on a grander scale.

Kenny and Buttel have observed that the "biorevolution" will affect many more crops and geographic areas than the Green Revolution.[41] Further, they foresee an important trend in how biotechnology will be disseminated. Because biotechnology will be controlled primarily by the private sector—as opposed to the Green Revolution which was largely driven by the public sector—the direction of biotechnology research and development will be to serve the needs of financially well-to-do sectors who can pay for the resulting products.[42]

Predictive studies of the economic impacts of increased agricultural production in developing countries provide a third piece of evidence suggesting that as new technologies are distributed in a society, the gap between rich and poor often widens. For example, Martha Gilliland has estimated the impacts of nitrogen-fixing biotechnologies on corn production in Mexico and on Mexico's economy.[43] Her conclusion is that the ultimate national economic effects depend primarily on whether the biotechnologies are implemented by the commercial agricultural sector, or whether the rural, small farm sector also has access. In particular, she demonstrates that a positive chain of effects would be initiated if

the technology were implemented in the rural sector, but not if it were implemented (as we would expect it to be) by the wealthier landowners.[44]

Gilliland's research reinforces the point that the outcome of any given technological gain is not a foregone conclusion predetermined by the science. It is mostly a function of the social and political systems involved. In fact, these social and political realities may profoundly affect the ultimate value of biotechnology research if they undo its potential beneficial effects. Thus, research scientists working on products for the benefit of developing countries, if they want their efforts to have the intended impacts, need to be cognizant of these problems.

Shifting Export Markets

A second economic risk associated with biotechnology is the risk caused by rapidly shifting export markets. In societies whose economies are contracting rather than expanding and which have pervasive infrastructure problems, most farmers will not be able to adopt biotechnology as soon as their counterparts in developed countries. Furthermore, it seems likely that research in biotechnology will permit industrialists and agriculturalists in the North to produce many of the final products and raw materials that were formerly available primarily from the South. This is likely to come about through the use of microbial engineering, fermentation, and use of new crop varieties that are adapted to temperate climates. Product substitution could further weaken the economic position of the South in international trade. Of course, the product substitution process has been going on for many years. However, with genetic engineering, substitutes can now be developed and adopted much faster than before, leaving less time for national economies to adjust and find new markets in which to compete.

It is useful to consider several examples. Product displacement from developing countries has already occurred in the sugarcane market. Cane was first replaced by sugar beets grown in the North, and now even this source has been replaced with corn sweetener, also grown and processed primarily in the North.[45] As another example, plant breeding advances have led to the development of a new substitute for rubber derived from the guayule plant. Rubber has historically been an important export crop of several African countries, but guayule is grown in Texas.[46]

While the two previous examples are already realities, they were accomplished not through genetic engineering but through classical crop breeding. Product substitutes will also result from genetic

engineering. For example, scientists are developing stress-tolerant crops to help overcome adverse growing conditions in developing countries. However, developments that will keep the industrial countries many steps ahead of the product advances made in developing countries can and probably will occur as well—but at a faster rate. Commercial incentives are the primary reason, but so is the fact that politicians in the North are engaged in a lively competition to get the most economic advantage possible from the new technologies. Consider one example: Frostban.[47] If Frostban succeeds in reducing frost damage on a wide variety of crops, we may see more tropical fruits grown in temperate climates such as the U.S. or Europe. The impacts of such a change on the export markets of developing countries, many of which are dependent on exports of tropical fruits and vegetables, are potentially quite serious. More important than the single example, however, is the overall trend which the example reflects. "[The] sad irony is that the United States Government lectures [developing] countries to get their economic houses in order and to strengthen their export sectors. . . only to have their efforts to do this wiped out by [competition from. . .] the same country that is lecturing them so severely."[48]

The fact of change in the global marketplace is not the problem here. Rather, it is the accelerated pace of change, made possible by genetic engineering, which may lead to particularly difficult economic problems for certain developing countries. The product substitution process is not unique to developing countries, of course—it will cause rapid market shifts within industrial nations as well. However, developing countries are likely to experience the largest number of lost or reduced market outlets and they are the least well equipped to provide the necessary job retraining and labor relocation that those losses imply.

The Technology Treadmill

Madden and Thompson have identified a related economic risk arising from biotechnology, which they call the "technology treadmill."[49] Their analysis shows that even under the positive assumption that some farmers can successfully adopt new biotechnologies before they are adopted on a wide scale globally, many of those individual farmers are likely to realize net losses as compared with the period before the new technology was introduced. They argue that sooner or later the technologies will be adopted globally, thus leading to higher overall production, worldwide price declines, and reduced profits except for those farmers who can expand the size of their operations sufficiently to compensate for the losses. Farmers unable to do so will have to seek employment in other economic sectors.

In the U.S., the demise of small farms is a controversial economic and social issue; in developing countries, especially if it occurs rapidly, it could have grim implications. Rapid displacement of large numbers of farmers to urban areas could add to already heavy unemployment, exacerbate environmental stresses and crime, and severely strain the few existing social services.

Again, the cause for concern is usually the lack of control over these displacements and their rapid pace, not simply the fact that they occur.

Recommendations

The preceding discussion suggests that a variety of primarily non-scientific issues need to be addressed if we are to succeed in our common goals of safeguarding the environment and providing economic opportunity for people in developing countries.

This paper does not review the status of current institutional mechanisms to address the three risks identified in this paper; that topic is important and needs further investigation. However, it is obvious that very few international research agencies or companies conducting genetic engineering research have implemented strategies to address these risks. Undoubtedly, one reason is that striking a responsible approach is not a simple task. It requires that we spawn and nurture institutions and processes that are capable of addressing these challenges. The task is daunting because of the multiple actors involved, too: research institutes in developing and developed countries, governments, private industry, international donors, and environmental and public advocacy groups.

What should be our immediate goals? First, we should certainly seek to further evaluate the three risks this paper has identified. Second, research institutions should ensure that governments of their countries are notified about environmental release experiments, and should comply with the highest possible international standards of safety. Third, institutions conducting research should develop internal procedures to ensure that the risks described in this paper are averted at least for the specific activities under their control. In this section, I consider these recommendations one at a time. I briefly describe the first two recommendations, but then concentrate on describing the third because I believe it is the most feasible, important and potentially effective.

Research on Economic Forces Affecting Biotechnology

To date, far too little research has been conducted to analyze the three risks described in this paper, namely:

- Whether economic and other nontechnical factors will undermine our ability to realize biotechnology's potential benefits,

- Whether incentives encouraging environmentally safe research and development are sufficient, and

- Whether (and which) major global markets are likely to be affected by genetic engineering and what will be the specific economic impacts on developing countries.

These questions deserve to be investigated in much more detail so that responsive and appropriate policies can be developed and pursued.

Safe Research and Use, With or Without Regulation

Whether or not specific laws pertaining to genetic engineering exist in a country where scientists plan to release engineered organisms, researchers should ensure that appropriate people in the host country government are notified of the deliberate release activity and given sufficient time to respond, and should ensure that their research meets the highest possible international standards of safety.

Where regulatory oversight does not exist in a developing country, finding the right authorities to notify is likely to be very difficult. Sufficient time for this must be built into the R&D scheduling process. To reduce the time that this will require in the future, the scientific community should encourage governments to develop clear procedures as quickly as possible; this is discussed further below.

In the meantime, however, research directors should ensure that work conducted under their supervision complies with the highest current standards of internationally recognized guidelines. Usually this will mean compliance with the intent of such guidelines rather than with the exact procedures they establish. For example, it is not feasible for a research scientist in Gambia to submit her/his experiment for review by the environmental ministry of another country. However, it should be feasible to arrange for an independent review of the research consistent with the basic standards that other countries have established.

Institutional Responsibility: Forming Appropriate Technology Assessment and Implementation Networks

The third and final step I wish to recommend is both the most feasible and most important for any individual research institution

to take. That recommendation is for research institutions to establish internal procedures for addressing the three types of risks described in this paper, as they affect the *particular* set of activities in which they are engaged. This applies regardless of whether the institution is a university-affiliated laboratory, an international agricultural research center, a multinational corporation, or an international donor agency.

The appropriate procedure for any given institution to adopt depends on its mission, organization and budget. Therefore, the following examples of how this could be accomplished are offered as illustrations rather than rigid recommendations for the kinds of approaches that can be envisioned. Research institutions and donor agencies can modify these ideas to suit their specific needs and circumstances.

Institutions need to begin by examining the types of activities they are conducting in light of the three risks described in this paper. This might be done by existing staff or with the help of consultants or an independent committee. The purpose of such an analysis is to help the institution understand which of these risks is most likely to threaten itsultimate goals.

Once the risks most relevant to a particular institution's work are identified, it has the information it needs to selectively hire a small, permanent staff whose job is specifically to help it address and avoid these problems. Committees of outside advisors, while useful for giving one-time advice, are almost always unable to devote sufficient time to implementing long-term solutions. Furthermore, permanent staff will have a much better understanding of the operating constraints and realities of the organization.

The types of staffs needed to address the issues being raised about the use of biotechnology products in developing countries will vary from one institution to the next, but one thing that should be clear from the issues reviewed in this paper is that technical training is not sufficient. Staff of such institutions should include at least some professionals who are trained in economic, social science, and policy disciplines—including but not limited to anthropology, economics, ethics, political science and other social sciences. The particular composition and size of such a staff must depend on the institution it is meant to serve.

While the suggestion to create this new function within research institutions may seem novel and untested, there are good precedents for it. Twenty years ago, most corporations involved in chemicals research did not have environmental staffs; now nearly all do, and

those staffs play an important role in helping the company achieve its goals while avoiding social and political backlash. One of the most important things they do is to provide advice about the R&D process from its inception, rather than letting the company get to a point where damage control is the only option. The same concept applies to research institutions involved in biotechnology research and transfer, even though the exact form and function of such staffs could and should be molded to the needs of the particu'ar organization.

For purposes of discussion, I will refer to these staffs as "Appropriate Technology Assessment and Implementation Networks," ATAINs. I deliberately choose to identify them as "networks" because even though ATAIN staffs might be very small (as small as one or two people at any given institution), their ability to address these issues effectively on behalf of the research community will be multiplied many times over if they are established in many such institutions. They could have an important role in advising research directors and research donor agencies about the issues raised in this paper, and related issues that may arise over time. Specific types of advice they could provide are suggested below.

One of the useful functions ATAIN staffs could perform would be to help their institutions become leaders in achieving the most important public benefits of biotechnology. For example, they could:

- Evaluate the social and economic merits of different research options and proposals.

- Design and implement methods to obtain information routinely and directly from intended beneficiaries of research, to ensure such research is meeting their needs.[50]

- Formulate standards and guidelines for addressing internal needs that encompass scientific and nonscientific functions of the institution. For example, ATAIN staff persons might work with their counterparts in other agricultural research institutions to develop model contracts for collaborative efforts between public and private research institutions.

To help encourage scientists to minimize environmental risk whenever possible, ATAINs could:

- Prepare guidance to help research scientists within the institution comply with environmental standards set by the host country or by international agencies.

- Determine in advance the institution's liability if something goes wrong under collaborative research arrangements in which it is involved, i.e., with private sector companies, developing country governments, etc.

Finally, ATAINs could suggest activities the institution could undertake to avoid possible negative impacts of research products.

- As one example, if a research proposal has great scientific merit but the ATAIN staff is concerned about the refraction effect, it could suggest innovative marketing strategies (including arrangements with private sector companies) that will ensure the resulting products reach poor, rural farmers.

In addition to helping their specific institutions more effectively address their own issues and needs, ATAINs could become an influential source of ideas for external changes that would benefit the research community by addressing some of the risks described in this paper. For example, to encourage *societal* development of the most beneficial public products of biotechnology, ATAINs could:

- Design model safeguards to preserve the independence of public sector research while building mutually beneficial collaboration between private and public sector research institutions.

- Conduct adaptive or applied research to modify research products and make them appropriate to local climatic, soil, market, and cultural conditions.

- Promote innovative programs for guiding research and development along the most needed channels within their countries; for example, by organizing national or regional scientist/farmer exchange programs and conferences, or by supporting "orphan products" legislation that would provide regulatory relief and perhaps economic subsidies to products that are important to society but otherwise would not be developed.

Second, ATAINs could encourage and contribute to the goal of designing biotechnology products having minimal environmental risks. For example, they could encourage and develop specific suggestions for implementing the following types of public policies:

- Effective regulatory capability within their countries.

- A requirement to use some proportion of the funds or profits from biotechnology research for research on ecological conse-

quences and risk assessment tools, by both the public and private sector.

- Development of common biotechnology safety approaches in international organizations such as the United Nations Environment Program (UNEP).

- Policies to teach nongovernment agencies that work in villages how to evaluate the environmental and nutritional value of new varieties and new practices.

- More funding for research on the underlying forces that may discourage scientists from using the safest R&D practices.

Finally, ATAINs could significantly further the goal of ensuring that biotechnology products have beneficial economic effects in developing countries. For example, they could develop and support:

- Deliberate policies in LDCs to ensure that biotechnology assists small as well as large-scale producers.

- Stronger public and private R&D in genetic engineering in developing countries. Such support could take the form of sponsoring scientific training, building scientific and professional societies, or supporting international efforts that are furthering this goal.

- Policies to mitigate the adverse effects of economic changes, e.g., strategies for coping with the risks of market loss, product displacement, and job displacement. Early Warning Systems to anticipate and monitor the social and distributional impacts of innovations could provide advance knowledge of the displacement of developing countries' products and markets by biotechnology applications.[51]

References

1. Frey, Kenneth, et al. 1985. "Genetic Improvement", in *Crop Productivity—Research Imperatives Revisited,* M. Gibbs and C. Carlson, eds., Report of an International Conference held at Boyne Highlands Inn, October 13-18, 1985, and Airlie House, December 11-13, 1985, p. 112.

   See also: Office of Technology Assessment. 1981. "Impacts of Applied Genetics: Micro-Organisms, Plants and Animals," Congress of the United States, Washington, D.C., p. 137.

2. Moss, J.P. and D.C. Sastri. 1988. "Use of Hormones and Ovule and Embryo Culture to Enhance Wide Crosses." Paper presented at the conference "Strengthening Collaboration in Biotechnology: International Agricultural Research and the Private Sector," Arlington, Va.

3. Shuh, G. Edward. 1987. "Foreword," *Notre Dame Journal of Law, Ethics and Public Policy,* 3:1, pp. 1-2.

4. Ibid, pp. 1-2.

5. Morehouse, Ward. 1985. Quotation of Francisco Sagasti in "The New Levers of Global Power: Science and Technology in the International Political Economy," *Third World Affairs,* Third World Foundation for Social and Economic Studies, London.

6. International Development Research Center. 1985. "Biotechnology: Opportunities and Constraints," Manuscript Report, Canada, pp. 70-72.
See also: Morehouse, Ward. Op cit., 1985.

7. McPherson, M. Peter. 1987. "Africa—an End to Famine," *Notre Dame Journal of Law, Ethics and Public Policy,* 3:1, p. 63.

8. Morehouse, Ward. Op. cit., 1985.

9. Doyle, Jack. 1985. *Altered Harvest: Agriculture, Genetics, and the Fate of the World's Food Supply,* Viking Penguin Inc., New York, pp. 94-113.

10. Abelson, Philip, ed. 1984. *Biotechnology and Biological Frontiers,* American Association for the Advancement of Science, Washington, D.C.
Battenfield, Susan, ed. 1983. "Proceedings of the National Interdisciplinary Biological Control Conference," held February 15-17, 1983, in Las Vegas, Nevada (published by the Cooperative State Research Service, U.S. Dept. of Agriculture, Washington, D.C.
Halvorson, Harlyn O. et al, eds. 1985. *Engineered Organisms in the Environment: Scientific Issues,* American Society for Microbiology, Washington, D.C.
Olson, Steve. 1986. *Biotechnology: An Industry Comes of Age,* National Academy Press, Washington, D.C.
U.S. Department of Commerce/International Trade Administration. 1984. "High Technology Industries: Profiles and Outlooks—Biotechnology," U.S. Government Printing Office, Washington, D.C.

11. Office of Technology Assessment. 1988. "New Developments in Biotechnology; Field Testing Engineered Organisms: Genetic and Ecological Issues," Congress of the United States, Washington, D.C., p. 76.

12. National Academy of Sciences. 1975. *Pest Control: An Assessment of Present and Alternative Technologies,* Vol. 1, Contemporary Pest Control Practices and Prospects, The Report of the Executive Committee, National Academy Press, Washington, D.C., pp. 161-162.
U.S. Environmental Protection Agency/Office of Pesticide Programs. 1976. "Incentives for Research and Development in Pest Control," National Technical Information Service, Springfield, Virginia, pp. 32-33.

13. Elkington, John. 1986. "Double Dividends? U.S. Biotechnology and Third World Development." World Resources Institute, Washington, D.C., p. 10.

14. International Development Research Center. Op. cit., 1985, pp. 70-82.

15. Ibid, p. 78.
See also: Johnson, Judith A. 1981. "Biotechnology: Commercialization of Academic Research," Library of Congress/Congressional Research Service Issue Brief No. IB81160, Washington, D.C., pp. 1-5.

16. Blumenthal, David, Michael Gluck, Karen S. Louis, and David Wise. 1986. "Industrial Support of University Research in Biotechnology," *Science* Vol. 231, 1/17/88, pp. 242-246.
See also: Olson, Steve. Op. cit., 1986, pp. 98-106.
See also: Kenney, Martin et al. 1982. "Genetic Engineering and Agriculture: Exploring the Impacts of Biotechnology on Industrial Structure, Industry-University Relationships, and the Social Organization of U.S. Agriculture," working paper prepared for the Workshop on Biotechnology, Agriculture, and the Public Interest, University of Kentucky, June 15-17, 1982, pp. 32-54.

17. Mooney, Pat Roy. 1983. "The Law of the Seed: Another Development and Plant Genetic Resources," *Development Dialogue* 1:2, Dag Hammarskjold Foundation, Uppsala, Sweden, p. 92.
See also: Krimsky, Sheldon, "Corporate Academic Ties in Biotechnology, *GeneWatch,* Sept.-Dec., 1984.

18. National Academy of Sciences Committee on the Introduction of Genetically Engineered Organisms into the Environment. 1987. "Introduction of Recombinant DNA-Engineered Organisms into the Environment: Key Issues," National Academy Press, Washington, D.C., pp. 8-9.
See also: Office of Technology Assessment. Op. cit., 1988, pp. 15-16.

19. Covello, Vincent T. and Joseph R. Fiksel. 1985. "The Suitability and Applicability of Risk Assessment Methods for Environmental Applications of Biotechnology," Final Report to the Office of Science and Technology Policy, Executive Office of the President, National Science Foundation, Washington, D.C., p. 2.
See also Levin, Simon A. and Mark A. Harwell. 1986. "Potential Ecological Consequences of Genetically Engineered Organisms," *Environmental Management* 10:4, p. 497.

20. Goldburg, Rebecca. 1988. "Environmental Concerns and Opportunities," unpublished speech delivered at conference entitled "Catching the New Wave: Biotechnology in Extension Programming," May 12-13, 1988, Michigan State University, East Lansing, Michigan.

21. Curtiss, Roy III. 1976. "Genetic Manipulation of Microorganisms: Potential Benefits and Biohazards," *American Review of Microbiology* 30, pp. 512-527.

22. Doyle, Jack. Op. cit., 1985, pp. 1-15.

23. Office of Technology Assessment/Food and Renewable Resources Program, 1986. "Staff Paper on the Role of the U.S. Development Assistance in Maintaining Biological Diversity in Developing Countries," Congress of the United States, Washington, D.C.
See also: Interagency Task Force Report to Congress. 1985. "U.S. Strategy on the Conservation of Biological Diversity," forwarded to the President of the United States under the signature of M. Peter McPherson, Administrator of the U.S. Agency for International Development, February 1985.
See also: Plucknett, Donald L., et. al., 1987. *Gene Banks and the World's Food,* Princeton University Press, Princeton, New Jersey.

24. The Conservation Foundation. 1988. "Opportunities to Assist Developing Countries in the Proper Use of Agricultural and Industrial Chemicals: Report of the Committee on Health and Environment," February 18, 1988, Washington, D.C., pp. 14-20.

25. Dasmann, Raymond F. et al. 1973. *Ecological Principles for Economic Development*, John Wiley & Sons, Ltd., New York, pp. 182-235.
See also: Miller, Taylor O. et al. 1986. *The Salty Colorado*, The Conservation Foundation, Washington, D.C. and the John Muir Institute, Napa, California, p. 6.

26. Mooney, H.A. and J.A. Drake. 1987. "The Ecology of Biological Invasions," *Environment* 29:5, pp. 10-37.
See also: Sharples, F.E. 1983. "Spread of Organisms with Novel Genotypes: Thoughts from an Ecological Perspective," *NIH Recombinant DNA Tech. Bull.* Vol. 6, pp. 43-56.

27. Goldburg, Rebecca. Op. cit., 1988.

28. Zaret, Thomas M. and R.T. Paine. 1973. "Species Introduction in a Tropical Lake," *Science*, Vol. 182, pp. 449-455.

29. "EPA's Novel Idea for a 'Suicide Plasmid', "*Biotechnology* 4:9, September, 1986, p. 762.

30. Office of Technology Assessment. Op. cit., 1988, pp. 13-15.
See also: "Detection Technology: The Key to Environmental Biotechnology," *BioTechnology* Vol. 4, May 1986, p. 419.

31. Drahos, David J. et al. 1986. "Tracking Recombinant Organisms in the Environment: Betagalactosidase as a Selectible Marker for Fluorescent Pseudomonads," *BioTechnology* Vol. 4, May 1986, pp. 439-444.
See also: "Release of Altered Microbes is Approved in Tracking Test," *New York Times*, October 10, 1987.

32. McGarrity, Thomas O. and Karl O. Bayer. 1987. "Federal Regulation of Emerging Genetic Technologies," *Vanderbilt Law Review* 36:3, pp. 476-478.

33. Edlin, Gordon J., "Biotechnology—A Regulatory Dilemma", undated paper.

34. Baumol, William J. and Wallace E. Oates. 1975. *The Theory of Environmental Policy: Externalities, Public Outlays, and the Quality of Life*, Prentice-Hall, Inc., Englewood Cliffs, New Jersey, pp. 56-69, 191-234.
See also: Schultze, Charles L. 1977. *The Public Use of Private Interest*, The Brookings Institute, Washington, D.C., pp. 29-46.
See also: Schumacher, E.F. 1973. *Small is Beautiful*, Blond and Briggs, London.

35. Center on International and Public Affairs. 1983. "Biotechnology and the Third World, A Round-Table Discussion: Notes on the Discussion," New York, September 15, 1983.
See also: "Regulatory Considerations: Genetically Engineered Plants", Summary of a workshop held at Boyce Thomson Institute for Plant Research at Cornell University, October 19-21, 1987, Center for Science Information, San Francisco, p. 17.

36. Slovic, Paul. 1986. "Informing and Education the Public About Risk," *Risk Analysis* Vol. 6, pp. 403-415.
See also: Yankelovich, Daniel, 1984. "Science and the Public Process: Why the Gap Must Close," *Issues in Science and Technology* Fall 1984, pp. 6-12.

37. Gilliland, Martha. 1987. "Nitrogen Fixing Biotechnologies for Corn in Mexico and Central America," *Impact Assessment Bulletin* 5:2, pp. 71-94.

38. Yotopoulos, Pan A. and Jeffrey B. Nugent. 1976. *Economics of Development: Empirical Investigations,* Harper & Row Publishers, New York, pp. 237-258.

39. Durant, Will, and Ariel Durant. 1968. *The Lessons of History,* Simon and Schuster, New York, pp. 52-57.

40. Buttell, Frederick H. et al. 1985. "From Green Revolution to Biorevolution: Some Observations on the Changing Technological Bases of Economic Transformation in the Third World," *Economic Development and Cultural Change* 34:1, pp. 61-91;
See also: Doyle, Jack. Op. cit., 1985. pp. 255-281.
See also: Morehouse, Ward, 1986. "Biotechnology and the Third World: Panacea or Recipe for Social Disaster?," Academy for Educational Development, Washington, D.C.

41. Kenney, Martin and Frederick H. Buttel. 1985. "Biotechnology: Prospects and Dilemmas for Third World Development," *Development and Change,* Vol. 16, p. 70.

42. Ibid.

43. Gilliland. Op. cit., pp. 71.

44. Gilliland's results are based on the assumption that corn production increases 50% in Mexico. She concludes that if increased productivity occurs in the rural, small farm sector, the country's balance of payments will increase 8.6%, gross domestic product will increase 1.5%, gobs will increase 1.5%, pressure to emigrate will decrease, and nutrition and food security will improve. She further concludes that if the productivity increases are realized only in the commercial sector, none of these advantages will be realized and certain socioeconomic problems will increase, like economic bifurcation.

45. Hobbelink, Henk. 1987. *New Hope or False Promise? Biotechnology and Third World Agriculture*, International Coalition for Development Action, Brussels, Belgium, pp. 23-25.

46. "Guayule: Natural Rubber from the Desert," *Agricultural Research* 32:14, Nov/Dec 1984, pp. 5-7.

47. Office of Technology Assessment. Op. cit., 1988, p. 125.

48. Schuh, G. Edward. Op. cit., p. 5.

49. Madden, Patrick and Paul B. Thompson. 1987. "Ethical Perspectives on Changing Agricultural Technology in the United States," *Notre Dame Journal of Law, Ethics, and Public Policy*, 3:1, pp. 93-94.

50. An example of such an approach is the one developed at the Centro Internacional de Mejoramiento de Maiz y Trigo (International Maize and Wheat Improvement Center) which integrates the farmer into the research process and considers interactions among agronomic, climatic, social, economic, cultural, and political factors that influence farm productivity.

51. Hobbelink, Henk. 1987. Op. cit., p. 57.
See also: McPherson, Peter M. Op. cit., p. 61.

Crop Disease Detection Through Biotechnology—Worldwide Applications

Richard K. Lankow
Agri-Diagnostics Associates
Cinnaminson, NJ 08077

Immunological Biotechnology

A major biotechnology area that has expanded greatly is the practical application of immunology-based techniques. The discovery of monoclonal antibodies in 1975 increased interest in immunology and has lead to a large number of research breakthroughs and a host of commercial products. The first applications of immunological biotechnology were in the medical area. Diagnostic products, diagnostic imaging, targeted delivery of therapeutic agents and direct therapy are among the prominent uses of monoclonal antibodies.

Immunological biotechnology has also found application in the agricultural realm. Uses of diagnostic products for veterinary medicine parallel those in human medicine. Diagnostic kits are available for many infectious diseases of domestic animals as well as for pregnancy and ovulation detection.

Crop plants have also become a focus for the application of immunological techniques. For some time plant virologists have led the way by utilizing animal-derived antibodies to detect, diagnose, and characterize plant virus infections in plants, seeds, and vectors. In recent years the application of immunological testing to plants has expanded a great deal due, in part, to the increased use of monoclonal antibodies and diagnostic technology in the medical fields.

Today immunological methods are being used both experimentally and commercially to detect and monitor crop diseases, detect and quantify pest populations, detect mycotoxins and pesticide residues, differentiate varieties, and identify biochemical components of plants.

Immunoassay Principles

The key to immunological testing is the nature and function of antibody proteins which are a key element of the defense mechanism of higher animals. Antibodies are produced by specialized blood

cells in response to organisms and high molecular weight substances which the animal's system identifies as "foreign." Antibody molecules have a consistent structural confirmation usually represented graphically as being similar to the letter "Y." The remarkable aspect of these proteins is that each contains a variable region which can bind to very specific materials (Fig. 1). This specific binding ability allows antibodies to recognize and attach to foreign materials encountered in an animal's blood stream or body as a critical step in the animal's defense mechanism. Foreign materials which can elicit an immune response are termed "antigens." When animals are immunized for the production of specific antibodies, the material used is termed an "immunogen." Mammalian immune systems are capable of generating a vast array of antibodies with different binding specificities. It has been estimated that the immune system may be capable of developing over one million different types of antibodies.

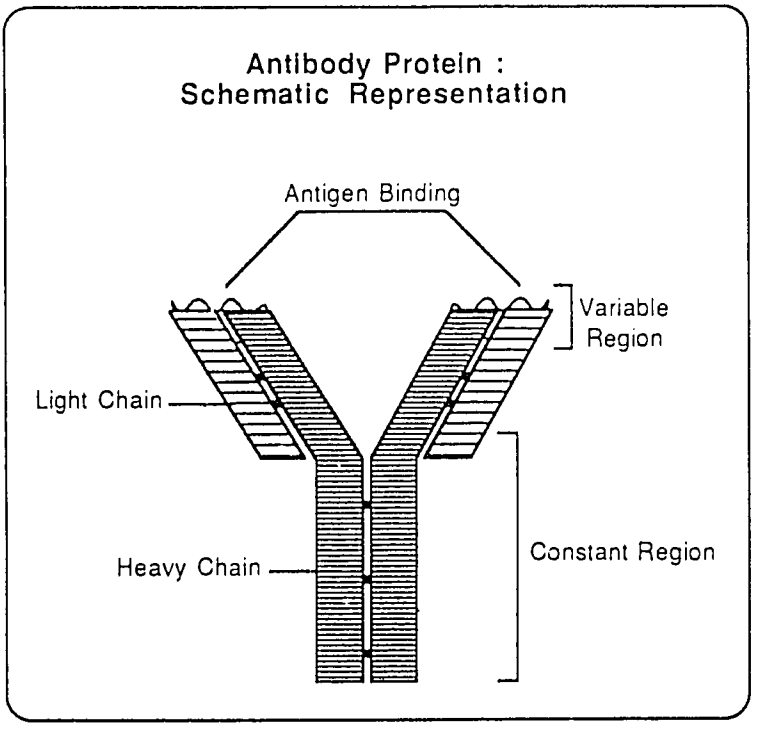


Figure 1. Antibody Protein: Schematic Representation.

Studies in the 1970s demonstrated that one white blood cell produces only one type of antibody. When that cell becomes stimulated it reproduces and secretes the specific antibody for

which it is genetically programmed. Since an animal is constantly stimulated by a variety of antigens the bloodstream contains many different antibodies at any one time. This multiplicity of antibodies can be problematical when the animal is being used to produce specific antibodies experimentally, since a variety of antibodies with differing specificities will be found in the blood serum along with antibodies of interest. Since each antibody is produced by a single cell population or clone and every animal produces many antibodies and cell clones, native serum is termed "polyclonal."

Polyclonal antisera are extremely useful and have been the basis of most medical immunodiagnostic testing for over 25 years. In general, polyclonal antisera display high affinity for the target-antigens and tend to exhibit fairly broad specificity ranges. With purification, polyclonal antisera can yield highly specific antibody fractions.

There are some drawbacks to polyclonal antisera including variations in the antibody population within an individual animal over time, the need to produce batches of immunogens regularly, and the purification required to increase the specificity of an antiserum.

In 1975 Kholer and Milstein demonstrated that single antibody-producing cells from mice could be isolated and grown in culture indefinitely, if the cells were physically fused with cancerous mouse white blood cells called myeloma cells. Myeloma cells have the capacity to reproduce in culture whereas normal white blood cells survive only a short time in culture. The resulting hybrid cells, "hybridomas," secrete antibodies, can be grown under laboratory conditions, and can be stored indefinitely under liquid nitrogen.

Through careful selection of immunogens and rigorous screening, researchers can develop monoclonal antibodies which exhibit exquisite specificity. Since these cells can be reproduced readily and stored easily, it is now possible to have consistent sources of antibodies. These characteristics have stimulated the commercial interest in monoclonal antibodies and immunologically-based testing since the basic discovery in 1975.

The specific binding ability of antibodies is used in immunological testing procedures in which antibodies are employed as highly specific reagents. In contrast, other analytical techniques derive much of their specificity from an apparatus or combinations of reagents and equipment. Analytical testing procedures which utilize antibodies are referred to as "immunoassays."

Immunoassays require a means to visualize or detect the reaction between an antibody and its target organism or substance to be

employed on a practical basis, and over the years many visualization methods have been developed. Since each antibody molecule has two binding sites, visible aggregates may be formed when an antibody reacts with target materials. Other assay methods have employed the ability of antibodies to induce lysis of red blood cells. The antibody molecule is notable in that it can be modified chemically without impairing its specific recognition and binding capacities. This characteristic allows the "tagging" of antibodies

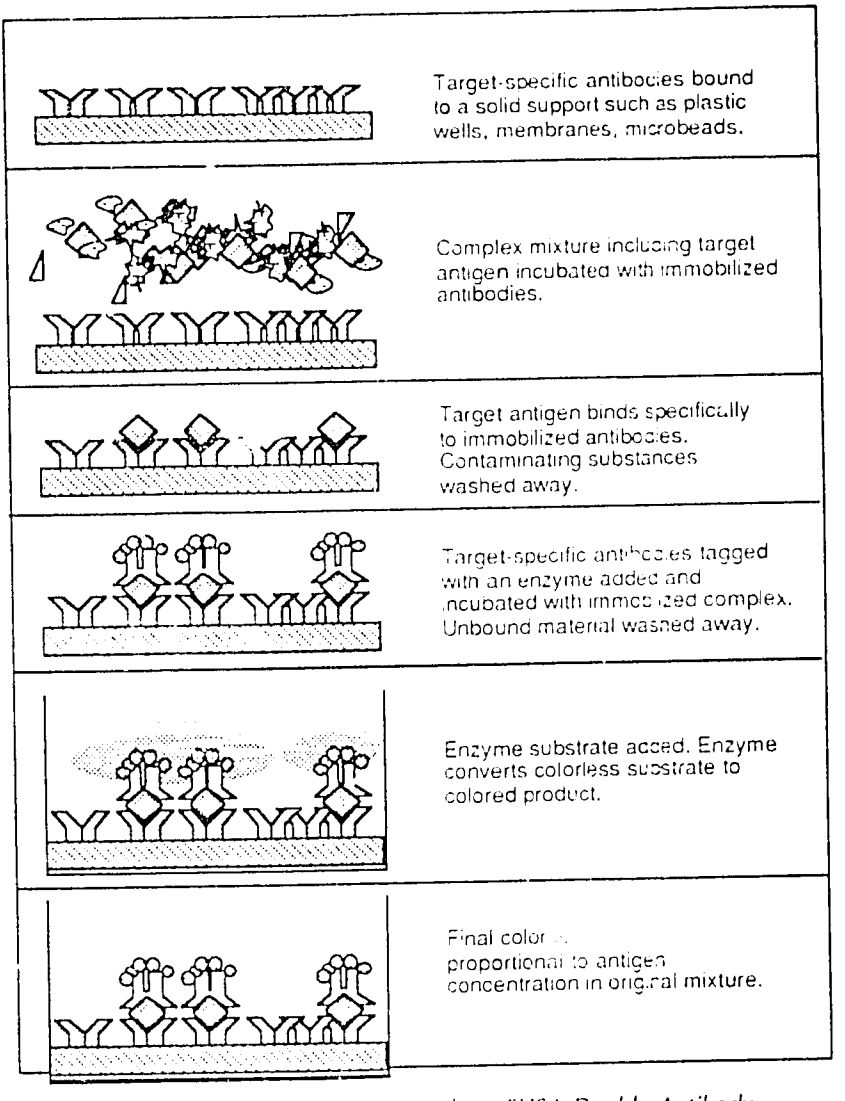


Figure 2. Schematic Immunoassay Procedure: ELISA Double Antibody "Sandwich" Assay.

with markers including radioactive substances, fluorescent materials, particles, and enzymes. The majority of immunoassays developed recently rely on tagged antibodies for visualization of reactions. In addition to visualizing reactions, the materials used to tag antibodies may also provide amplification which increases the sensitivity of the assay.

One of the most popular and effective visualization methods utilizes enzymes which are biochemically coupled to the antibody. Enzymes are chosen for their ability to catalyze a reaction in which a colored end product is generated. This type of assay is termed "EIA" for enzyme immunoassay or "ELISA" for enzyme-linked immunosorbent assay. Figure 2 outlines a typical ELISA test. Although there are many types and variations of immunoassays, this example offers an overview of the steps of a typical immunoassay.

Immune systems can generate specific antibodies to a vast range of materials. The practical exploitation of this requires that one select the target to be detected, develop antibodies which recognize that target, and formulate the antibodies and associated reagents in a useable assay. Viruses were among the first plant-associated materials to be targeted for antibody production. In recent years, however, antibodies have been produced which target a wide variety of plant components, pathogens, and pests.

Immunoassays in Crop Production

The applications of immunoassay in medicine are well-known and growing, but the applications in crop agriculture have been less clear until recently as plant disease detection and diagnosis products have become more widely available. Commercial tests are available for a variety of plant viruses, a limited number of plant pathogenic bacteria, and several important genera of plant pathogenic fungi.

Immunoassays are especially suited to the detection and identification of specific organisms in crude preparations. Due to the binding specificity of antibodies, target organisms can be detected in plant extracts, seed homogenates, soil preparations and other such matrices without the need to isolate the pathogen. Immunoassays can be developed to detect extremely low levels of the target and can be quantitative. These characteristics make immunoassays applicable for use in laboratories in offices or homes, and at field locations.

Crop Disease Management

Crop yields have risen under intensive cultivation but pests and diseases continue to claim a significant portion of agricultural production. Consequently, intensive crop production schemes require active disease management programs to assure high and consistent crop yields.

Goals of disease management programs include:

- Reduction of disease losses
- Prevention of catastrophic crop loss due to disease
- Appropriate uses of pesticides and other inputs
- Maximum economic yield.

The objectives of disease management systems required a high level of observation including scouting for disease outbreaks, assessing planting materials for freedom from infection, assessing the degree of disease potential in the environment, and attempting to anticipate disease outbreaks. These tasks are especially difficult in the case of plant diseases, since most pathogens are microscopic and the presence of the pathogen can be observed only when symptoms appear or when very laborious and lengthy pathogen isolation procedures are employed. Detection and diagnosis of plant diseases typically requires a well-trained and equipped individual. Detailed information on pathogen level can normally be obtained only through the use of a well-equipped laboratory.

Biotechnology and Crop Disease Detection

The techniques of immunoassay discussed above can greatly augment the process of crop disease detection and diagnosis. Although the development of immunoassays for crop diseases is highly sophisticated, the assays and products arising from those efforts can be relatively simple, which allows their use in less sophisticated settings. The driving force in the commercial development of immunological assays is their application to nonlaboratory settings worldwide.

Detection of plant pathogens through the use of immunoassays has a wide range of implications and benefits. A number of applications of immunoassays and the use of the resulting information are discussed below.

Crop Disease Diagnosis: Growers, crop managers, and technical specialists throughout the world must diagnose crop diseases regularly. Aside from the high level of training and experience

required, this task is made more difficult by the variation in disease symptoms, similarity in symptoms from different causes, and secondary pathogens. Laboratory diagnosis, when it is available, may be equally difficult since samples frequently have deteriorated before they reach the laboratory. Most diagnostic laboratories are relatively far from growing regions which further limits the availability of sophisticated diagnostic services. These problems are amplified in developing countries where limited transportation and mail services may make laboratories more inaccessible.

Immunological methods of pathogen detection overcome many of these limitations. Due to their great specificity, immunoassays may frequently be applied to crude plant extracts that contain plant components and perhaps a variety of secondary organisms and pathogens. Once a specific pathogen has been detected in a plant sample, appropriate control measures can be developed and implemented.

It must be emphasized at this point that immunoassays can not make disease diagnoses. These assays and any other method can only assist the crop manager in developing a specific diagnosis. For example, the detection of a pathogen in a sample must be weighed against the symptoms being expressed, recent weather patterns, the crop variety being grown and so forth. The accurate detection of pathogens is, however, a critical aspects in the proper diagnosis of crop diseases.

Once accurate detection of a pathogen has been accomplished and the disease diagnosed, the crop manager can implement control measures. The benefits of accurate, rapid, and simple disease diagnosis include:

- Timely management practices
- Appropriate management action
- Wider range of management options

The sensitivity of immunoassays allows the detection of crop disease prior to the onset of symptoms. Pathogens present in seed and other propagating materials, latent foliar and fruit infections, and active root infections can be detected before symptoms are expressed or become well-defined. Early detection of diseases and pests is a clear goal of all pest management systems for reasons summarized in the accompanying table.

- Increases management options available
- Apply most appropriate disease control
- Select most appropriate planting materials

- Limit disease spread
- Prevent crop losses

Disease detection is critical throughout the crop production cycle. Selection of disease-free planting materials and early detection and diagnosis of diseases are critical to sound disease management. Similarly, disease levels must frequently be monitored throughout the growing season. In this way crop management decisions based on changes in disease levels or frequency and control practices can be adjusted accordingly. Benefits of regular monitoring include:

- Accurate timing of disease management practices
- Additional detail on disease patterns
- Appropriate and effective use of fungicides

Ideally crop disease management should also be based on knowledge of pathogen prevalence before planting or even before planting sites are chosen. Currently this information is developed for a very limited range of plant pathogens which are primarily seed-borne or carried in asexually propagated materials such as tubers, bulbs, rootstocks, and woody species. The level of disease inoculum in the environment is very difficult and has typically been determined almost exclusively on a research basis.

Characteristics of Crop Immunoassay Systems

The prior sections have outlined the nature of immunoassays and have outlined the needs for crop disease monitoring. Immunoassays can contribute greatly to disease monitoring and diagnosis in agriculture, but the assays themselves must be adapted to fit into agricultural systems. Since the initial commercial uses of immuno-muno-assays were in clinical medicine, most procedures required a well-equipped, temperature-controlled laboratory staffed with well-trained personnel.

Agricultural immunoassays, in contrast, must frequently be conducted near the growing site to be of practical benefit. Diagnostic laboratories will continue to expand their use of immunoassays but such facilities are, unfortunately, not readily accessible to growers and crop managers even in highly developed countries. Agriculture presents a number of challenges to developers of immunoassays that are not encountered in medical diagnostic test development.

Development of Commercial Immunoassays

Medical	Agricultural
Single host (Humans)	Large number of host species
Host well characterized	Limited biochemical characterization
Pathogens well characterized	Limited biochemical characterization
Sampling: blood, urine, biopsy	Sampling: must be developed
Diagnosis by individual	Diagnosis and detection in populations
Diagnostic system established: Physician, Clinical reference labs, Hospital labs, Home diagnostic test kits, Laboratory technicians	Diagnostic system limited: University, state diagnostic labs, Private diagnostic labs
Tests conducted in controlled environment.	Tests conducted under varying conditions
Effect of body substances on tests known.	Effect of plant, soil extracts not known

Despite the challenges inherent in developing immunoassay to agricultural targets, many research groups, both academic and commercial, have succeeded in creating useful antibodies and assays for plant pathogens. At the point that useful antibodies have been developed, they must be assembled into a testing format that meets a number of criteria. Many of the immunoassay criteria listed below must be met for medical diagnostics, but a number are unique for agricultural immunoassays.

Criteria for Commercial Agricultural Immunoassays

- Test standardized, results reproducible
- Can not employ radioactive labels
- Testing rapid, requires limited hands-on action
- Sample preparation simple, rapid
- Multiple samples can be tested
- No interference from host plant materials
- Quantitative or yes/no assay
- Cost-effective (testing cost relative to treatment and potential crop loss)
- Can be conducted in nonlaboratory setting
- Test results meaningful—interpretation guidelines established

In general, agricultural immunoassays can be grouped into on-site and laboratory-type tests based on the sites where they will be used. On-site testing will be conducted at or very near the growing site. These tests will be analogous to over-the-counter medical testing and will comprise the major use of immunoassays due to the inaccessibility of most laboratories as discussed above. On-site testing imposes additional criteria on the design and development of commercial diagnostic products.

Criteria for On-site Agricultural Immunoassays

- Test units self-contained
- Specialized laboratory equipment not required
- Test procedures simple and explained clearly
- Tests relatively insensitive to environment
- Interpretation guidelines and subsequent actions defined

It should be noted that immunoassays are dependent on biological reagents including antibodies, enzymes, and proteinaceous stabilizers. These materials must be stored under controlled conditions to preserve their full activity. Most test kit manufacturers specify storage under refrigeration when kits are not in use. These limitations must be considered when diagnostic assays and kits are targeted for specific crops and regions of the world.

Implications for International Agriculture

Commercial Status: Currently, there are a number of commercial immunoassays available to detect and diagnose plant diseases. The great majority of these are laboratory tests targeted to virus diseases. AgDia, Inc. and Boeringer-Mannheim have the most extensive product listings. Agri-Diagnostics Associates produces laboratory immunoassays for several genera of plant pathogenic fungi. Immunoassay kits for aflatoxins and other mycotoxins are available from a number of firms throughout the world. Environmental Diagnostics, Inc. markets a 10-minute screening assay for aflatoxin. Laboratory immunoassay for aflatoxin are currently available for Neogen, Inc. (US), May and Baker (UK), and Ube Industries (Japan).

On-site tests are currently available for several fungal diseases from Agri-Diagnostics Associates. A number of other firms have announced additional on-site tests but these are still under development.

Development of New Diagnostic Products: International agricultural agencies and research centers must become involved in a number of key activities if commercial diagnostic tests are to be available on a worldwide basis. These are outlined below and include identifying crop diseases for which diagnostic tests are truly needed and determining the priorities for their development. Many times a product may be needed by a particular group of growers or specialists in a particular region, but upon further study it is found that the problem is limited in its distribution and importance. International agricultural experts and agencies are in the best position to provide direction to commercial firms regarding target selection.

It is also critical to define the management action to be taken once a positive test is obtained. What will the technical specialist or grower do if they discover the presence of a particular disease in a crop or seedlot? If no clearcut management action is apparent, the test, though technically successful, may not contribute to management of the crop. Similarly, the economics of the management action must be considered in relation to the value of the crop and the cost of the diagnostic procedure.

The term "biotechnology" implies a commercial aspect of biological research and development. International agricultural agencies must participate actively in assisting biotechnology companies to define the economics of disease problems and their control in the international community. These aspects include support of development of specific tests, licensing agreements with basic researchers who may have developed antibodies and techniques, and developing distribution networks for the resulting testing products.

New technologies require end-user education and training. This concept applies to the United States and other developed countries as well as to the international agricultural community. If the products of biotechnology are to benefit international agriculture, customer education and training is a challenge that will require the commitment and involvement of all agencies and specialists. Collaborative efforts are essential for the successful commercialization of any new technology. In agriculture this is doubly true due to the vast areas involved, the diversity of crops and environmental conditions, language barriers, trade policies, and the need to educate end-users.

Key Steps In Diagnostic Kit Development

- Identify target diseases in various regions and crops
- Set priorities for development of diagnostic reagents
- Define the management steps resulting from a positive test
- Determine where and by whom field tests will be conducted
- Arrange funding for field testing
- Identify end-users
- Determine who will purchase and distribute tests
- Arrange for end user education and training

The Future: Although immunodiagnostic products can be developed relatively quickly, the development process requires a sophisticated and integrated team of specialists from diverse disciplines including immunology, protein biochemistry, plant pathology, agronomy, and bioengineering. The need for integrated teams may limit the rate at which additional diagnostic products will reach the market place.

Biotechnology is being driven forward primarily in the United States and other developed countries; consequently, international agricultural agencies and policy making groups must develop guidelines and priorities which identify the needs that biotechnology can address. Once the goals are clearly set, biotechnology research groups and commercial organizations can begin to bring the enormous power of this group of techniques into the battle for stable, cost-effective agricultural production worldwide.

Selected References

The following list provides a cross section of references relevant to agricultural immunoassays. More exhaustive bibliographies can be found in the articles by Lankow *et al.*, Miller and Martin and Halk and DeBoer.

Barret, J.H. "Textbook of Immunology," C.V. Mosby Company; St. Louis, 1983.

Halk, E.L. and DeBoer, S.H. *Ann. Rev. Phytophthol.*, 1985; 23, 321-350.

Kabat, E.A. "Structural Concepts in Immunology and Immunochemistry," Holt, Rinehart, and Winston, 1976.

Kohler, G. and Milstein, C. *Nature* (London), 1975, 256, 495.

Lankow, R.K., Grothaus, G.D., and Miller, S.A. In: "Biotechnology In Agricultural Chemistry: ACS Symposium Series 334," American Chemical Society; Washington, D.C.; 1987.

Miller, S.A. and Martin, R.R. *Ann. Rev. Phytopathol.*, 1988, 26 (In Press)

Ngo, T.T. and Lenhoff, H.M. "Enzyme-Mediated Immunoassay," Plenum Press: New York, 1985.

Notton, B.A., Fido, R.J., and Galfre, G. *Planta*, 1985, 105.

Pestka, J.J., Li, Y., Harden, W.O., Chu, F.S. *J. Assoc. of Anal. Chem.*, 1981, 64, 294-301.

Stern, N.J. and Gamble, H.R. "Hybridoma Technology in Agricultural and Veterinary Research," Rowman and Allanheld Totowa, New Jersey. 1984.

Weiler, E.W. *Physiol. Plant.*, 1982, 54, 230-234.

Weir, D.M. "Handbook of Experimental Immunology," Volume I; Immunochemistry. Blackwell Scientific Publications: Oxford, 1978.

Wie, S.I. and Hammock, B.D. *J. Agric. Food Chem.*, 1982, 30, 949-957.

Immunoassay Technology for the Diagnosis of Disease in Livestock and Developing Countries

Erwin F. Workman, Jr.
AgriTech Systems, Inc.
Portland, Maine

Veterinarians have long recognized the need for rapid and accurate diagnosis in sound health care programs. Over the years, a wide variety of diagnostic technologies have been employed in response to this need. Most recently, advances in biotechnology, including the advent of immunoassay technology, have ushered in an exciting new era of improved diagnostic capabilities for a broad range of animal health applications.

Tests that were once cumbersome to perform or difficult to interpret can now be done quickly, easily, and accurately with minimal skill requirements in a veterinary clinic or in the field. High-volume automated diagnostic systems have also revolutionized testing procedures in veterinary laboratories. The impact of these new diagnostic capabilities on animal health management is far-reaching and certainly deserves the close attention of practicing veterinarians around the world.

The purpose of this presentation is to provide an overview of immunoassay technology as it is applied in the laboratory and in the field. In order to demonstrate the breadth of the technology, examples of immunoassays for three different diseases, each performed using different assay technologies, are presented. These assay technologies include a state-of-the-art, automated, fluorescence-based system, a standard microwell system, and finally, a single-use assay device appropriate for field use.

As these disease detection systems are presented, keep in mind that the particular diseases chosen may not seem appropriate to everyone. But remember that they are merely examples to give you an appreciation for the capability of the technology, so feel free to make mental substitutions as you read through this presentation.

Before reviewing immunoassay technology, it is useful to briefly review the immune response. As shown in Figure 1, when an individual is challenged with viral or bacterial antigens, this "foreign material" is first processed by the macrophages. This involves breaking up the material into fragments which are presented in association with the major histocompatibility complexes to the T-lymphocytes (T-cells). The T-cells are subsequently activated and

stimulated to divide. Note that the interleukins play a role in T-cell activation. B-lymphocytes (B-cells) also interact with the foreign antigens as a function of receptors different from those on T-cells. B-cell receptors are known as immunoglobulins. Subsequent to an interaction with antigen, activation of the B-cells is completed via stimulation by other lymphokines secreted by the T-cells. The activated B-cells then undergo cell division producing B-cell clones. Each clone is capable of producing a specific immunoglobulin recognizing one site or epitope on the viral or bacterial antigen. These B-cell clones then mature into plasma cells whose function is to produce and secrete immunoglobulins into the blood. These antibodies circulate throughout the body and are capable of binding to foreign antigens. This binding triggers additional mechanisms which process and neutralize the foreign antigen.

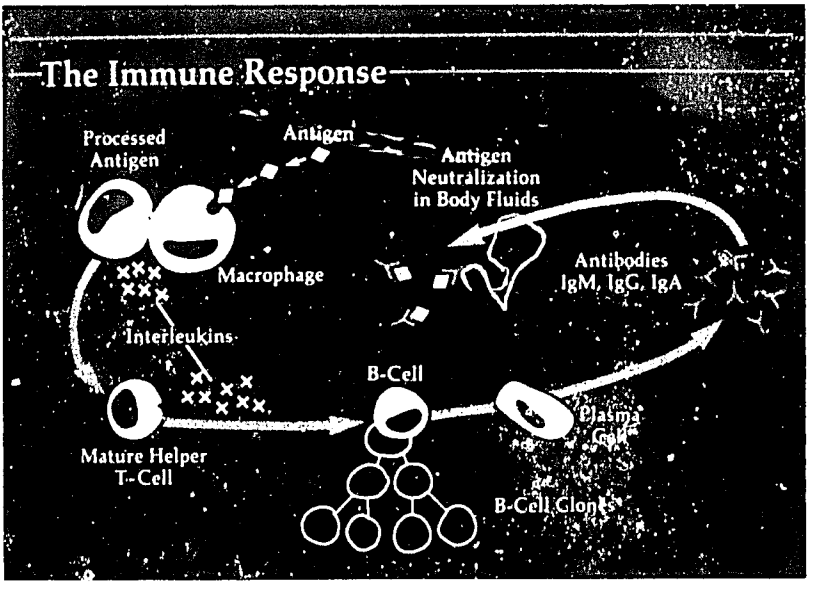


Figure 1. Simplistic view of the immune response.

We take advantage of the immune response in diagnostics not only for the production of diagnostic reagents, both monoclonal and polyclonal antibodies, but also for the detection of antibodies that are markers of disease. There are three basic requirements for an immunoassay. First of all, reagents are needed; antibody if the goal is to detect specific antigen, or purified antigen if the goal is to detect antibody. Secondly, a matrix for the antigen-antibody reaction to occur is required. A microtiterplate would be an example of a matrix, as well as the dipstick. Finally, a readout system to measure the extent of the antigen-antibody reaction is needed.

The three assay technologies that will be discussed are presented in Figure 2. In the middle is a standard microtiter plate. In the background may be seen the multiwell reaction vessel for the automated, fluorescence-based system. Finally, in the foreground, you see a single-use assay device.

The fluorescence-based system is presented in Figure 3. It consists of the instrument on the left and a computer on the right. Basically, the instrument performs the entire immunoassay, then sends data to the computer for reduction. The technology is called PCFIA, or Particle Concentration Fluorescence Immunoassay. It utilizes microscopic particles or submicron particles, which serve as the matrix for the antigen-antibody reaction. These particles are coated with reagents and are used to bind reactants. Bound and unbound labels are separated by filtering the reaction mixture. The machine can filter because it pulls a vacuum across the bottom of the wells, pulling the liquid through but retaining the microparticles on top of the membrane. After washing, fluorescence is read by the instrument. Figure 4 presents a cross-section of the wells. Microparticles may be seen in suspension. After the reaction is complete, the mixture is filtered, and the particle washed and concentrated on the bottom of the well. The total particle-bound fluorescence is then determined by front-surface fluorimetry. It is the concentration of particles on the membrane that gives the technology good sensitivity.

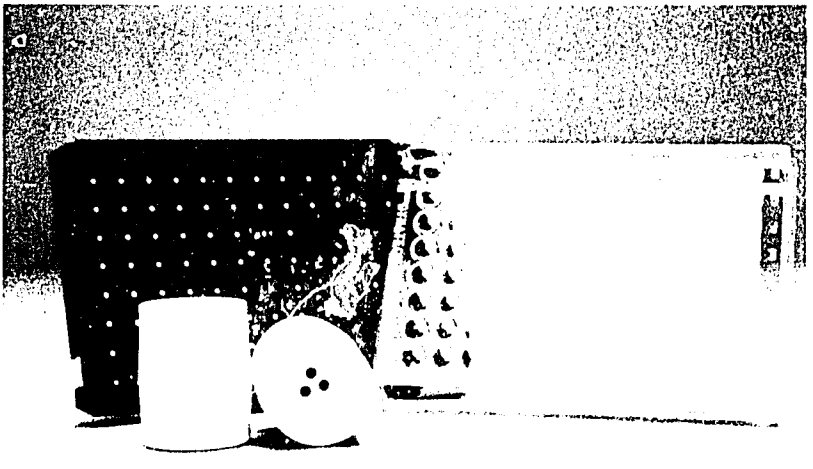


Figure 2. Three different assay delivery technologies.

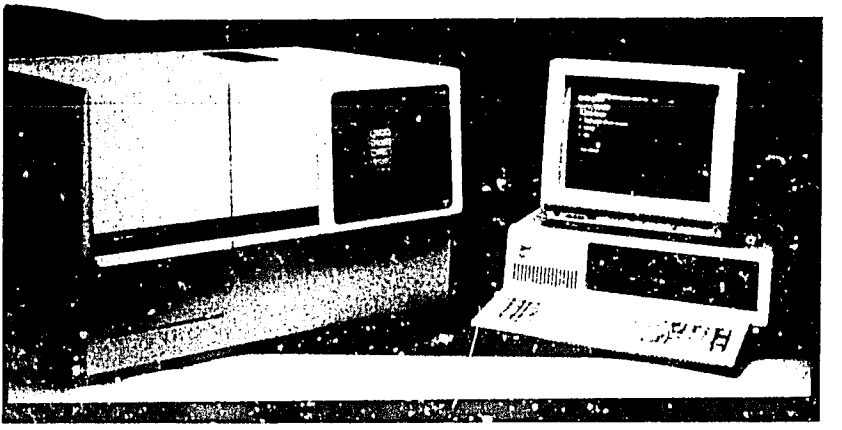


Figure 3. PCFIA system.

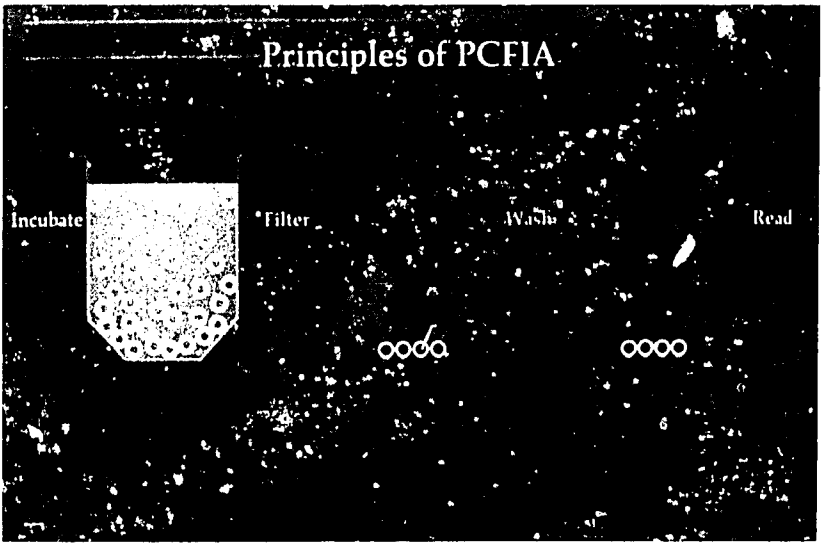


Figure 4. Cross-section of PCFIA reaction wells.

The disease example for this technology is brucellosis. The test system is designed to detect antibodies to *Brucella abortus* in bovine serum, and has been set up in a competitive assay format. The microparticle is coated with antigen from *Brucella abortus*, and is mixed with sample and with the label. The label is a fluorescene-labeled antibody specific for *B. abortus*. Antibody in the sample competes with the label for binding sites on the microparticle, such that the more antibody in the sample, the lower the fluorescence. To perform this test, the technician fills up a reagent reservoir, which is inserted into the instrument. Then, sample is added to each of the reaction wells in each of ten plates. Next, the plates are inserted in the instrument and GO is pressed on the touch screen.

The instrument does the rest. It adds the antigen-coated particles, adds the fluorescene-labeled antibody, and proceeds through the incubation and wash steps. Finally, it measures fluorescence, and sends the data to the computer.

As shown in Figure 5, one technician and one instrument can run about 3000 samples a day. That extrapolates to about three quarters of a million samples per year. If one adds technicians to handle the sample information, then the throughput goes up and, of course, if instruments are added, then the throughput goes up.

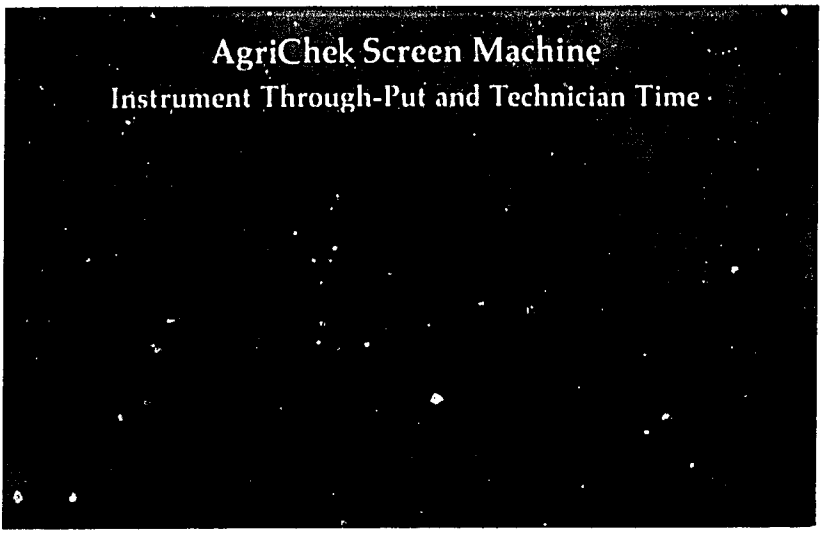


Figure 5. PCFIA throughput for a B. abortus antibody test.

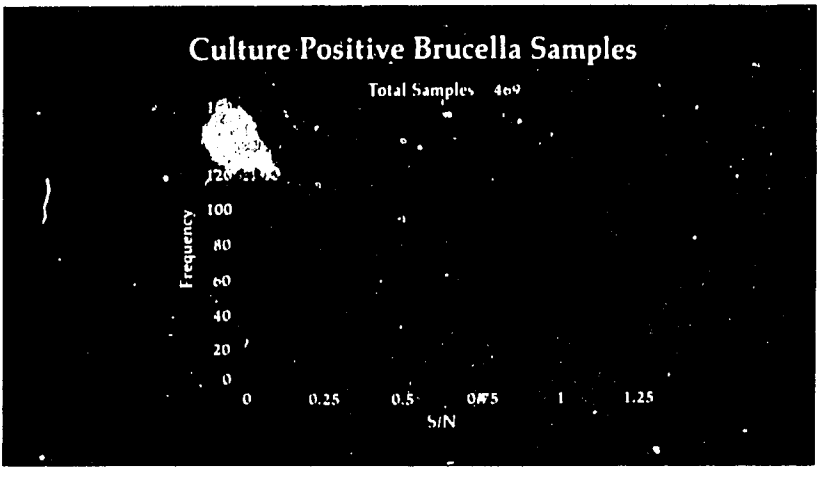


Figure 6. Frequency distribution for B. abortus culture positives using PCFIA.

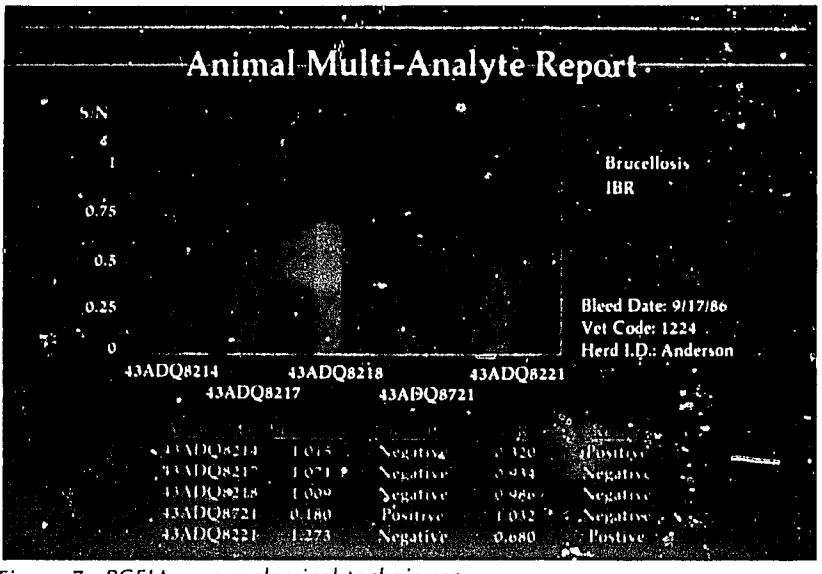


Figure 7. PCFIA versus classical techniques.

To give an idea of the sensitivity of this methodology, a frequency distribution on 469 culture-positive brucella samples is presented in Figure 6. Frequency versus a sample to negative ratio, S/N, is plotted. Ratios of 0.7 or below are considered positive, while those greater than 0.7 are considered negative. In this case, 466 of the 469 samples are positive. Compared to classical methodologies (Figure 7) PCFIA is equivalent or better.

In terms of specificity, which is the ability of a test to call a negative sample negative, PCFIA is superior to the current presumptive tests. Figure 8 presents 29,000 samples tested in Pennsylvania, a brucellosis-free state. If all samples are assumed to be negative in this free state, any sample with an S/N that is 0.7 or below then would be a false positive. From this distribution it is apparent that the false positive rate is very low. In fact, it is on the order of 0.3 percent. How does that compare with current methodologies? Probably the most popular presumptive assay for brucellosis is called BAPA, a plate agglutination test. Twenty-two thousand samples were tested by both PCFIA and BAPA in Pennsylvania. The BAPA methodology called a total of 360 of those samples positive, whereas PCFIA called only 89 positive. Thus, the BAPA screening method has about four times the false-positive rate compared to PCFIA. Late in 1987, this methodology was approved as an official testing methodology for brucellosis in the United States, and we think it will have a positive impact on eradicating the disease.

In addition to high throughput of sensitive and specific assays, the technology has additional beneficial attributes. These include the capability of measuring fluorescence simultaneously at up to four different wavelengths. This means that if one can configure multiple assays in the same format, adding in multiple particles with different antigens and multiple probes with different fluorophores, then one should be able to perform multiple assays on the same sample simultaneously. In Figure 9 a multianalyte report is presented that was generated on samples that were tested for brucellosis as well as infectious bovine rhinotracheitis. A S/N of 0.7 or below would be positive for each disease; various combinations are presented.

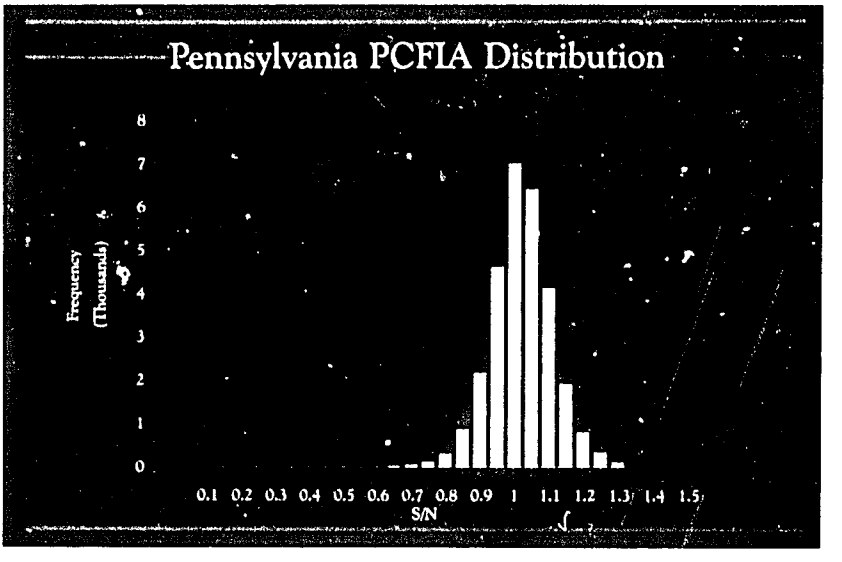


Figure 8. Frequency distribution for B. abortus negatives using PCFIA.

This is a very powerful technology. In addition to brucellosis and IBR, AgriTech is pursuing the development of assays for tuberculosis, Johne's disease, bovine leukemia, swine brucellosis, swine pseudorabies, and some other respiratory diseases. The Company would be interested, as well, in collaborating with developing countries to apply this technology to other diseases.

Microwell technology is the most commonly used assay technology. Consequently, around the world one will find a variety of readersystems and wash systems; there are both automated and manual versions. Manufacturers include Dynatech, Biotek, Flow, and Molecular Devices.

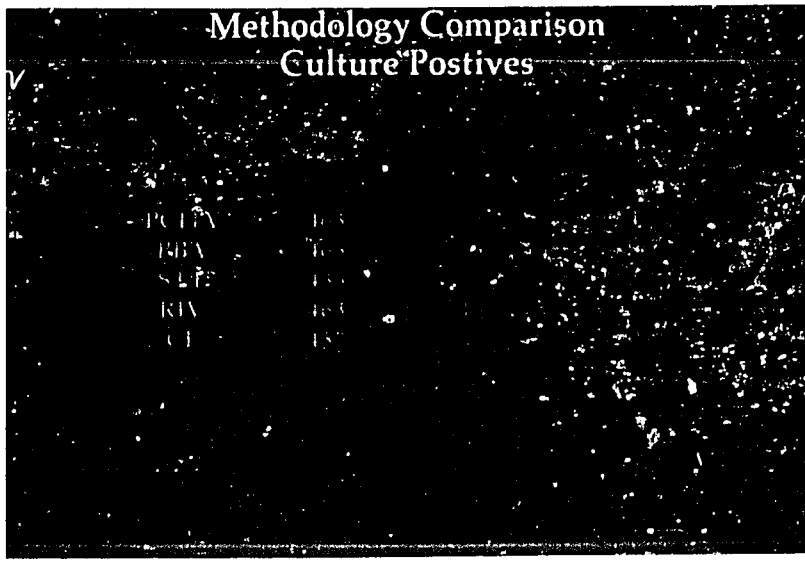


Figure 9. PCFiA multianalyte report.

The disease example for microwell technology is pseudorabies. The assay to be described is an assay that will differentiate a field-infected animal from an animal that has been vaccinated with a genetically engineered vaccine. On this program AgriTech has collaborated with another biotechnology company called SyntroVet, Inc. This Lenexa, Kansas-based concern has engineered a virus such that virulence factors, as well as a nonessential glycoprotein, gpX, have been deleted from the genome. Thus, a field strain of the virus will have gpX, whereas the engineered vaccine strain will not have it. Consequently, animals exposed to field strain will develop antibodies to gpX, but vaccinated animals will not. This is the basis for a differential diagnosis.

AgriTech developed monoclonal antibodies against gpX and set up a competitive enzyme-linked immunosorbent assay (ELISA), represented schematically in Figure 10. In this format the enzyme-labeled monoclonal antibody competes with gpX-specific antibodies in the sample. The more antibodies present in the sample, the lower the color.

What are the basic criteria for a differential test? First, it must identify field infections with the same sensitivity as a whole virus test, that is, a test based on detecting antibodies against all viral components. And, of course, it has to ignore all the antibodies produced in animals that are vaccinated with the engineered virus.

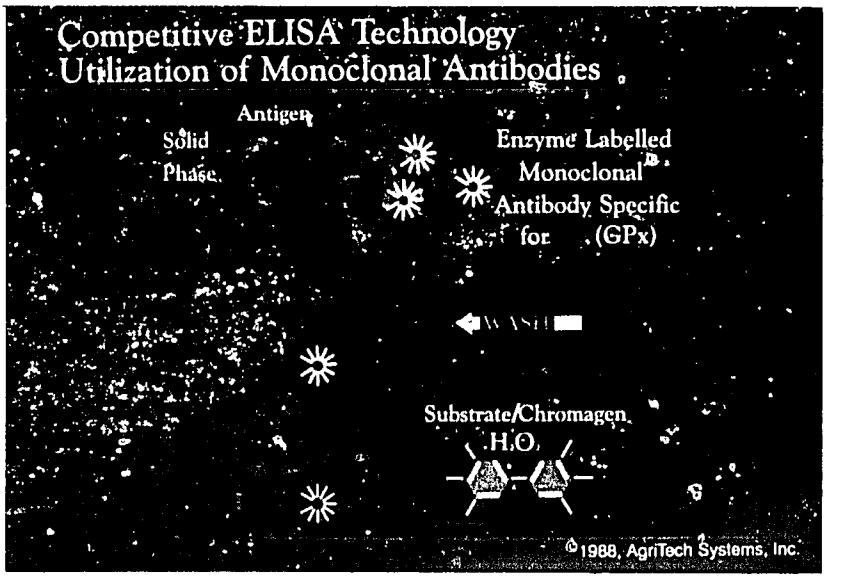


Figure 10. Competitive ELISA for anti-gpX.

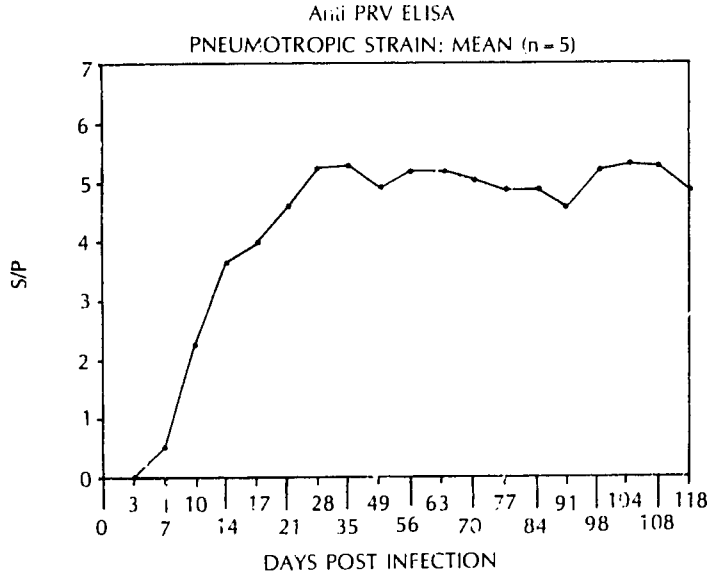
Performance of the gpX assay compared to the whole virus assay on infected animals is presented in Figure 11. Whole virus assay results are shown in the top panel; gpX assay results are shown in the bottom panel. On day 7 the whole virus test turns positive and as well on day 7 the gpX test is positive, indicating that the sensitivity of the gpX test is essentially equivalent to that of the whole virus test.

Figure 12 presents the performance of the whole virus assay and the gpX assay on vaccinates. The top panel indicates that titer is being developed subsequent to vaccination, seroconversion occurring at about day 10. On the bottom panel one sees the results on the same animal for the gpX assay. There is absolutely no response. It is clear that this assay can be used to differentiate field infections from animals that have been vaccinated with the SyntroVet vaccine.

AgriTech considers the microwell technology to be a very good one. As a matter of fact, the company has about two dozen products in the microwell format, including about a dozen tests for poultry diseases, two whole virus tests for pseudorabies, and tests for food and feed contaminants.

New assay technologies are typically applied first in laboratory environments and only later in nonlaboratory settings. This is certainly true for ELISA technology, which has become commonplace in most veterinary reference laboratories, but has only more recently

been adopted as a practical in-clinic diagnostic tool. AgriTech's goal in terms if in-clinic and field applications was to deliver im-



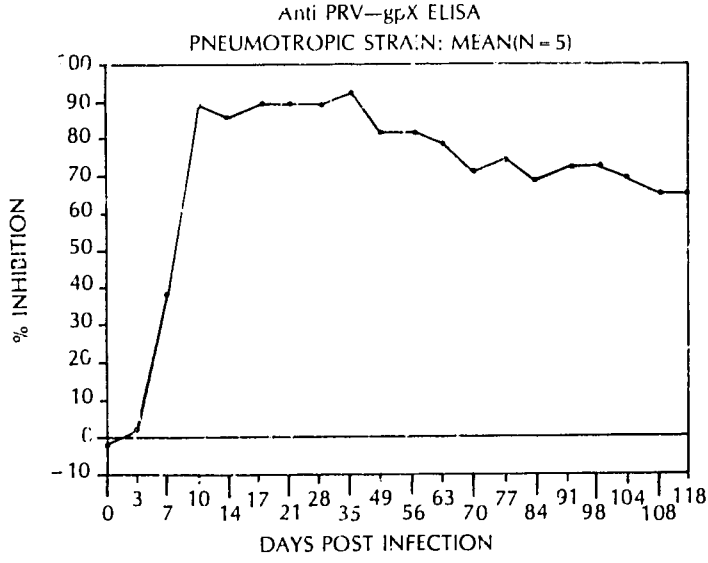


Figure 11. Comparison of sensitivity for the anti-gpX assay versus the anti-whole virus assay.

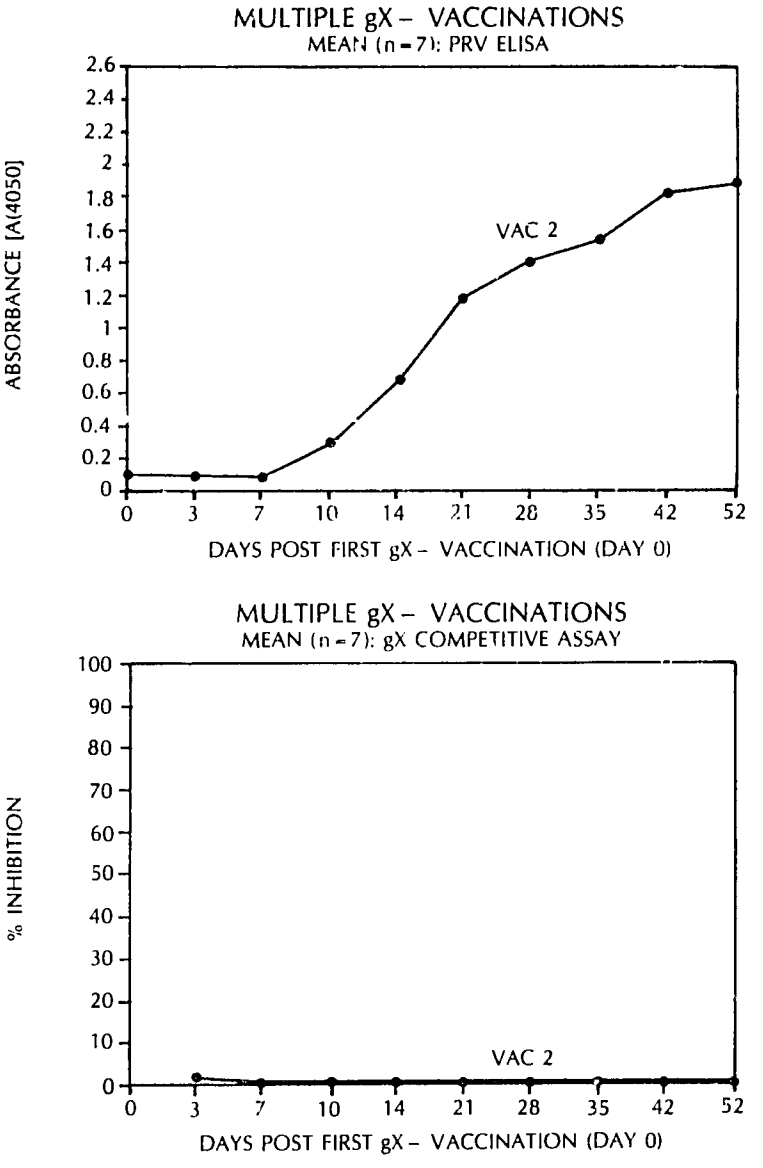


Figure 12 Comparison of the anti-gpX assay to the anti-whole virus assay on vaccinates.

muncassay technology to these groups without compromising sensitivity and specificity. We thought of using the microwell format, but decided that it should be utilized in the laboratory which is equipped with readers and washing systems. Thus, we turned to a completely new assay delivery technology called concentration immunoassay technology, now known throughout the veterinary clinic world as CITE.

This membrane filtration technology is presented diagrammatically in Figure 13. All of the immunoassay reactions occur on a glass fiber membrane. On top of the bioactive membrane is a prefilter. As a sample is applied onto this prefilter, it removes potentially interfering insoluble material such as blood cells and insoluble parts of fecal samples. The sample then moves across areas on the bioactive membrane where biological reagents have been immobilized. Past that, it moves into an absorbent area or waste reservoir.

Figure 14 shows a schematic of the device without a prefilter. Notice that there is ample space for multiple reactive spots. This allows the incorporation of integral positive and negative controls.

The disease example for the CITE technology is feline AIDS, which is caused by a newly discovered retrovirus, feline T-lymphotropic lentivirus. This virus causes an immune deficiency syndrome in cats. Virus is grown in tissue culture, and highly purified. The assay protocol contains five steps and is simple and quick.

A typical positive result is presented in Figure 15. Notice the orientation spot that indicates that below is the positive control. As long as that positive control develops color, it means the assay was performed correctly. Off to the right is a diagnostic spot. Here we have immobilized the FTLV antigens; thus, in this particular sample there were specific antibodies directed against that antigen.

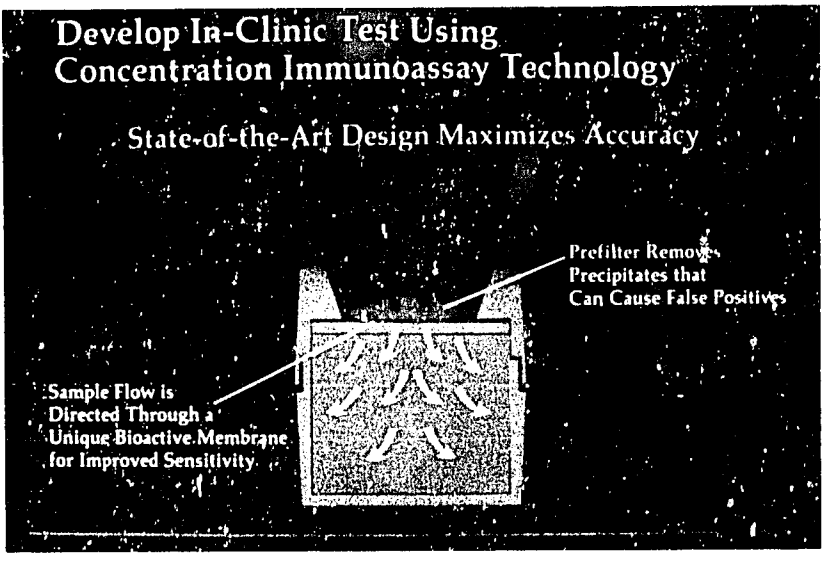


Figure 13. Membrane filtration technology.

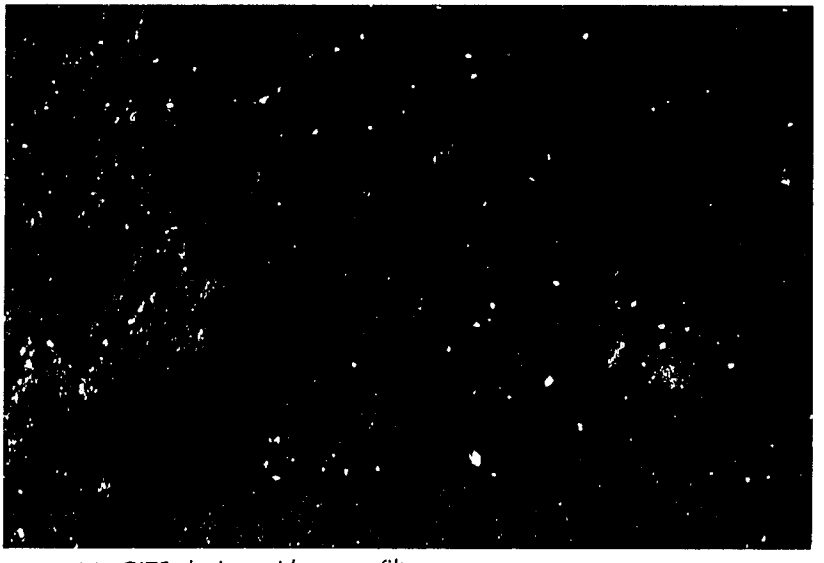


Figure 14. CITE device without prefilter.

It is possible, in the case of FTLV, for cats to develop an antibody titer against the cell line that is used to produce the virus. The incorporation of a negative control on the bioactive membrane which contains host cell components signals the presence of nonspecific reactivity. Since each sample serves as its own negative control, the chances for false positives as a function of nonspecific reactivity are virtually eliminated. An example of nonspecific reactivity is shown in Figure 16.

It is safe to say that any assay that functions on PCFIA or in the microtiter format can be adapted to the membrane filter format.

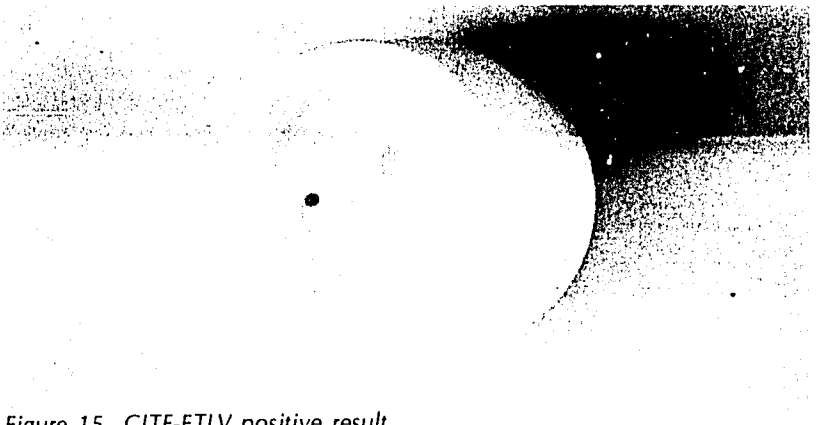


Figure 15. CITE-FTLV positive result.

Figure 17 presents an example of a brucellosis test that is being used at sale barns as a supplementary test on card-positive, vaccinated animals. In this example, the spot directly beneath the orientation spot contains the brucella antigen. In addition, calibrators are provided and, as the color develops in the sample spot, these calibrators also develop color. The level of antibody in the calibrators are matched to the various cutoffs in the PCFIA B. abortus test. Negative and reactor samples are shown in this figure.

In summary, diagnostic technologies have evolved rapidly over the last few years partly as a function of developments in biotechnology. In fact, technologies exist to provide any user anywhere in the world with a practical immunoassay system. The key, however, is to try to understand the needs of specific users and to understand the cost-benefit ratios that exist. Meetings such as the A.I.D.-sponsored conference on biotechnology provide a useful forum for users and manufacturers to exchange ideas and discuss opportunities.

CITE™ NON-SPECIFIC REACTIVITY

Figure 16. CITE nonspecific reactivity.

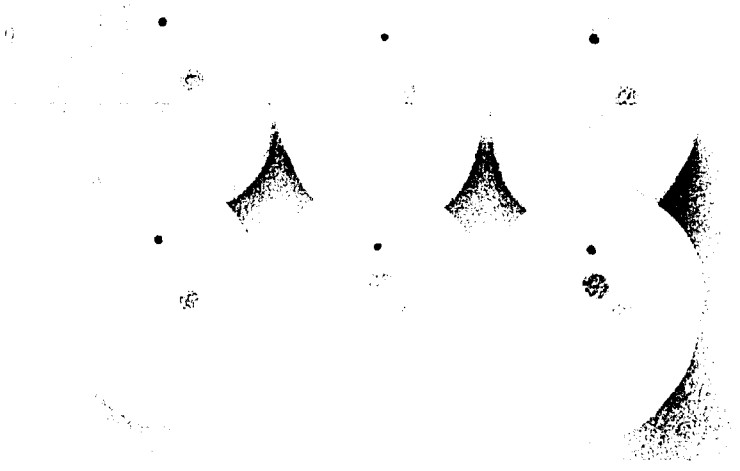


Figure 17. CITE—B. abortus test results.

Applications of Immuno and DNA Hybridization Diagnostics in Research at ICARDA

Khaled Makkouk, Douglas Beck and Marlene Diekmann
International Center for Agricultural Research in Dry Areas (ICARDA), Aleppo, Syria

Introduction

During the last few years, diagnosis of cereal and food legume viruses and identification of nitrogen fixing *Rhizobium* of food and forage legumes at ICARDA's main station in Syria and in the countries of its outreach (West Asia and North Africa, which will be referred to in this article as the WANA region) was based mainly on serology and biological indexing. Polyclonal antisera produced in rabbits were the main reagents used for detection and the techniques employed were mainly enzyme-linked immunosorbent assay (ELISA) for viruses and immunofluorescence (IF) in addition to ELISA for the detection of rhizobia.

The utilization of polyclonal antisera in many cases proved to be useful in detecting viral pathogens, including those which are seed-borne. However, in cases such as bean leaf roll virus (BLRV), the detection with polyclonal antisera did not appear to be sensitive and specific enough to explain the variability in disease symptoms observed in the field. The availability of more specific and sensitive diagnostic reagents will improve our understanding of the etiology of such diseases. The recently developed monoclonal antibodies with high sensitivity (Prohbakar et *al.*, 1984) and variable specificity are an added tool to improve diagnostic capabilities, not only for plant viruses and rhizobia, but may be extended to fungal and bacterial pathogens as reported recently (Casano et *al.*, 1987; Mitchell, 1986; and Mitchell and Sutherland, 1986).

The more recently developed molecular hybridization techniques employing complementary DNA (cDNA) probes for the detection of plant viruses and viroids proved to increase detection sensitivity (Owens and Diener, 1981; Macquarie et *al.*, 1984; Hull, 1984). Techniques were also developed to detect dsRNA in plant extracts (Morris et *al.*, 1983) as an indication of viral infection. Plant pathologists and microbiologists have at their disposal different detection methods with high sensitivity and reliability. Such innovations are important for seed health laboratories dealing with quarantine pathogens. The increased sensitivity of available methods will reduce the chances of seed-borne pathogens escaping laboratory

seed health testing. This is useful for a center such as ICARDA, which is actively involved in germplasm exchange.

Disease Diagnosis

Diagnosis of Virus Diseases

During the last few years, surveys for viral diseases of cereals and food legumes carried out in the WANA region (Makkouk *et al.*, 1987; Makkouk *et al.*,1988) identified a number of viruses either of actual or potential importance. On cereal crops, barley yellow dwarf virus is wide-spread in the region (Makkouk *et al.*, 1987), but it reaches economic levels only in Tunisia and Morocco, and possibly in Ethiopia. The surveys carried out are so far by no means complete and further work could identify other countries of the WANA region where cereals are seriously affected with BYDV. Identification of BYDV was based mainly on insect transmission and serology, where polyclonal antisera were employed by using either direct or indirect ELISA procedures (Makkouk *et al.*, 1987). Results obtained so far indicate that the PAV type (transmitted by the aphid species *Rhopalosiphum padi* and *Sitobion avenae*) of BYDV is the most common in Jordan, Morocco, Syria and Tunisia.

More recently, newly developed monoclonal antibodies specific for the PAV, MAV and RPV types of BYDV were also employed in our tests. Results obtained with these monoclonals also indicated that the PAV type, nonspecifically transmitted by aphids, was the most common in all the countries surveyed. In addition to the PAV type, RPV (transmitted specifically by the aphid *R. padi*) was detected in Algeria, MAV (transmitted specifically by the aphid *S. avenae*) in Morocco and RPV + MAV in Jordan, Syria and Tunisia. In these tests two different monoclonals each for PAV, MAV and RPV were used, one prepared against a U.S.A. isolate (S. Wyatt, WSU, Pullman, Washington) and the other against a U.K. isolate (L. Torrance, MAFF, Hatching Green, Harpenden). Still around 25% of the BYDV isolates tested did not react with any of the monoclonals used, but reacted with a polyclonal antiserum type F from Bioreba, Switzerland. For survey purposes, BYDV monoclonals with broader specificity are needed to make virus surveys more accurate as was found for polyviruses (Jordan and Hammond, 1987). If such monoclonals are not identified, a mixture of two or three monoclonals could serve the purpose. Work published recently suggests that two types of monoclonals could be identified; those with narrow specificity, useful for typing isolates and those with broad specificity, useful for field surveys.

Food and forage legumes are known to be far more susceptible to viral infections than cereals. Surveys carried out in faba bean fields in a number of countries of the WANA region identified nine viruses which infect the crop (Makkouk *et al.*, 1988). Bean leaf roll virus (BLRV) seems to be the most important followed by bean yellow mosaic virus (BYMV), broad bean mottle virus (BBMV) and broad bean stain virus (BBSV). BLRV is also the causal agent of "chickpea stunt" disease. BLRV should be regarded as an important pathogen affecting faba bean and chickpea in the WANA region.

Observations in the region over the last few years suggest that viruses could be more important on faba bean, chickpea and lentils than is recognized and some of the diseases diagnosed as *Fusarium* wilt or root rot could be in fact virus-induced. BLRV, a luteovirus concentrated mainly in the phloem vessels, interferes with the translocation of nutrients to the root resulting in poor root growth. Faba bean plants inoculated with BLRV produce a weak root system often black in color. It is likely that this disease syndrome results from attack of many secondary organisms which are usually harmless to healthy plants.

The identification of the virus or viruses which produce bean leaf roll or chickpea stunt symptoms are by no means complete. At present it is based mainly on the symptoms produced and on successful transmission by aphids in the persistent manner. There could exist a number of different luteoviruses which share the above characteristics. Recently, the provisional identification of chickpea viruses in California suggested the involvement of three different luteoviruses (I.W. Buddenhagen, personal communications). Chickpea was reported earlier in California to be infected with legume yellows (Duffus, 1979). Such a virus could be the same or related to lentil yellows observed recently in Ethiopia (Beniwal, personal communications). More work on the etiology of the luteoviruses affecting legumes in the WANA region is needed.

The already developed polyclonal antisera for BLRV (Ashby and Hutt'nga, 1979; Hampton, 1983) reacted weakly with BLRV isolates from the WANA region. The development of specific monoclonal antibodies for the luteoviruses affecting faba bean, chickpea, and other legumes will add significantly to our understanding of these viruses and the diseases they induce. Such information has consequences for breeding cultivars resistant to these viruses.

DNA probe technology has proved over the last few years that it has the potential of greater sensitivity with equal speed when compared to ELISA for the detection of plant viruses (Dunez, 1987). cDNA radioactive probes have been prepared for BYDV in private

and public laboratories (Gerlach, personal communications). Such probes are very useful for ICARDA's work on BYDV and also to its collaborators in the region. Since many laboratories are not equipped with facilities to assay radioactivity, however, biotin (Leary *et al.*, 1983) and photobiotin (Forster *et al.*, 1985) derivatives incorporated into the cDNA probes are more useful for routine diagnostic labs such as the ICARDA virology lab. In addition, monoclonal antibodies specific to modified nucleotides incorporated into virus cDNA probes were also recently developed (Tchen *et al.*, 1984). Specific cDNA probes (which contain the modified nucleotide sequence) could be detected by using enzyme-labeled monoclonals capable of recognizing the modified nucleotide. Such approaches could increase the sensitivity of detecting plant pathogens of cereal and food legume at ICARDA in leaves, roots or seeds.

Diagnosis of Seed-borne Pathogens

The use of sensitive and reliable methods for the detection of seed-borne pathogens is vital for any seed health laboratory. The development of sensitive immunodiagnostic techniques such as ELISA permitted the testing for seed-borne viruses in seed groups of 50-200 seeds rather than testing of single seeds. The establishment of tolerance levels of seed-borne viruses of 1:10,000 or even higher is practical when sensitive detection techniques are available. The availability of monoclonal antibodies for some viruses increased further the sensitivity of the test.

The classical biological testing for fungal pathogens in seeds is space and time consuming, as well as costly. For the detection of seed infection rates below 0.5%, the number of seeds to be tested should be more than 1000. This is not feasible when seeds have to be tested singly on agar media or blotter paper. However, if seed groups could be tested with sensitive immunodiagnostic reagents for seed-borne pathogens, a quicker, less costly and possibly more precise diagnosis will be possible.

The first report on the use of monoclonal antibodies for the detection of fungal pathogens was by Iannelli *et al.* (1983), where *Fusarium* spp. were successfully identified. In the years that followed more fungal-specific monoclonals were produced. Mitchell (1986) and Mitchell and Sutherland (1986) used monoclonal antibodies to identify *Sirococcus strobilinus*, a seed-borne pathogen causing shoot blight in pine, spruce and fur seedlings. In seed testing, less than 25 ng of fungal protein could be specifically detected by employing an indirect ELISA. There are also successes

in producing monoclonals for bacterial pathogens. Casano *et al.* (1987), recently reported the production of monoclonal antibodies specific for three pathovars of *Pseudomonas syringae*.

The use of monoclonal antibodies specific to fungus species could be of great help in differentiating between important and less important pathogens that are otherwise difficult to identify. Differentiation based on morphologic characteristics of the spores is not an easy task and requires some experience. Examples of interest for the ICARDA seed health laboratory are *Tilletia* spp. and *Fusarium* spp., *T. caries* and *T. foetida* which are endemic in the region, whereas *T. contraversa* which has not been reported in Syria. In seed health testing of cereals and legumes *Fusarium* spp. are detected frequently. Many of them have a low pathogenic potential or are saprophytes, others, such as *F. oxysporum* on legumes are of great importance. The identification of *Fusarium* species is time consuming and a reference collection is needed.

Table 1 shows the seven seed-borne viruses presently of interest to ICARDA programs with testing for these viruses being done by polyclonal antisera and by direct ELISA. The quality of the antisera permits the testing of seed groups of 20-200 depending on the individual virus. The availability of monoclonal antibodies for viruses with broad specificity and higher sensitivity will increase our precision and testing capabilities.

Production of monoclonal antibodies against a synthetic double-stranded RNA (Benhamou *et al.*, 1987) which can detect various dsRNA molecules even in trace amounts in infected tissue could have many applications in plant pathology. Such monoclonals are useful for detecting the replicative form of any seed-borne RNA virus in germinating seeds. Even though a technique such as this is not virus-specific, there is the capability of detecting virus-infected seeds, which is the main purpose of a seed health laboratory. In cases where the virus needs to be identified, as in the case of quarantine pathogens, further testing could be carried out only on those samples which were positive when tested by the nonspecific dsRNA monoclonals.

Plant-Microbe Interactions

Detection of Rhizobia

For practical purposes, *Rhizobium* bacteria are classified according to cross-inoculation groups, based on their ability to form effective symbioses with the different legume species. Strain specific qualities are associated with the ability of these bacteria to nodulate and ef-

Table 1. Seed-borne Pathogens of Primary Importance to the Seed Laboratory of ICARDA.

Pathogen		Crops
(a)	Fungi	
	Pyrenophora spp. (3 species)	barley
	Fusarium spp. (about 10 species)	cereals, legumes
	Tilletia spp. (4 species)	wheat
	Ustilago spp. (3 species)	wheat, barley
	Urocystis tritici	wheat
	Ascochyta spp. (4 species)	legumes
	Botrytis spp. (2 species)	faba bean
(b)	Bacteria	
	Pseudomonas pisi	pea
	Xanthomonas translucens	cereals
(c)	Viruses	
	barley stripe mosaic virus	Cereal crops, especially barley
	bean yellow mosaic virus	faba bean, lentil, pea, *Vicia sativa* and other forage legumes
	broad bean mottle virus	faba bean, lentil
	broad bean stain virus	faba bean, lentil
	broad bean true mosaic virus	faba bean
	pea seed-borne mosaic virus	Pea, faba bean, *Vicia sativa*, *Lathyrus odoratus* and other forage legumes
	pea enation mosaic virus	*Lathyrus odoratus*, pea

fectively fix N_2. The ecology of rhizobia in the soil, and especially in the rhizosphere where critical interaction takes place, it clearly crucial to the establishment and performance of the legume symbiosis. Information on the ecology of these microorganisms is a component of the goal of enhancement of biological nitrogen fixation (BNF).

Rhizobia have a narrow niche in nature, the legume root nodule, but need not depend on that niche for survival. Rhizobia introduced into a soil maintain a population base often independent of any opportunity to occupy a narrow niche. Therefore, their behaviour as free-living bacteria must be a major determinate of success in nodulation.

The use of autecological techniques in the past decade has provided ample evidence that soil rhizobia are adept at becoming rhizosphere rhizobia. The most pressing problem in the ecology of *Rhizobium* centers is the matter of competition, since some indigenous strains regularly outcompete both other indigenous strains and more desirable inoculant strains for nodule occupancy. The attributes that contribute to the success of an invasive strain are unknown, but must be elucidated if the potential of the most effective N-fixing rhizobia are to be exploited. In some cases inoculant strains are competitive, and are not only able to replace the indigenous populations in the nodules but also become established in the soil.

For model-system investigation of rhizobial competitiveness, ability to distinguish strains from one other is necessary. The interpretation of data from field legume inoculation trials is assisted by the capacity to determine the relative proportions of root nodules occupied by both introduced and indigenous types. Selection of superior strains by the best inoculation method depends on the ability to show that a specific strain occupies a significant number of nodules.

Mutation of a strain for antibiotic resistance has been used as a marker (Brockwell et *al.*, 1977), but careful testing of a mutant is required to determine that it is genetically equivalent to the original strain in symbiotic characters. Use of immunofluorescent staining or an enzyme-linked immunosorbent assay (ELISA) to detect rhizobia in crushed nodules may be useful (Fuhrmann and Wollum II, 1985; Dughri and Bottomley, 1983), despite the fact that it may be nonspecific due to cross-reactivity of rabbit antisera (Vincent, 1982). Adsorption of polyclonal antiserum with cross-reactive strains is a useful technique to obtain more specific antiserum (Olsen and Rice, 1984), but also may be cross-reactive with similar strains.

Polyclonal antibodies developed at ICARDA have proved to be valuable in developing sets of highly effective strains for regional chickpea and lentil inoculation trials, where inoculant strains are distinguishable from native populations and one another using ELISA techniques. In cooperation with ICARDA, regional scientists are able to determine where the need exists to inoculate these crops, and to select highly effective competitive strains prior to an inoculation effort. Research concerning the efforts of biotic and abiotic stresses on nodulation and N *in situ* fixation is also made possible through the use of fluorescent antibody and ELISA techniques. Classifying regional strain types may be possible using serological and plasmid identification techniques, providing valuable information about the origin of these legume species. Where limitations exist in the use of polyclonal antisera for separation of strains, it may be feasible to produce monoclonals for specific requirements (Wright *et al.*, 1986).

Plant Pathogens

Breeding for disease resistance to several types of plant pathogens (viruses, fungi, bacteria) is based on evaluating plant reaction to infection. Jedlinski *et al.*, (1977) demonstrated a direct relationship in oats between virus content and tolerance to infection with barley yellow dwarf virus (BYDV). The development of the ELISA technique permitted the quantification of viral content in plant extracts (Hammond *et al.*, 1983). Skaria *et al.* (1985), reported that for some virus/host combinations tested, symptomatic resistance to BYDV as previously determined by plant breeders was associated with reduced viral productivity in infected plants, and this effect was cultivar-specific and virus isolate-specific. Tentative results at ICARDA using a number of barley, bread and durum wheat lines showed a high correlation between symptomatic resistance upon infection with a PAV-type BYDV and average ELISA values at 7, 8 and 9 weeks after inoculation for barley low in both bread and durum wheat. A BYDV monoclonal antibody (MC32-39) provided by S. Wyatt was used in these evaluations. Further testing of breeding lines is needed before generalizing such results. The more recent use of cDNA probes for PAV or RPV types of BYDV (Lorens *et al.*, 1987) shows promise for studying the interaction between BYDV and cereal breeding lines. The commercial availability of such probes would facilitate the work for BYDV resistance in many research centers including ICARDA. Such an approach would also prove useful for studying other virus/host interactions once the virus-specific probes become available.

Another area of interest for crop improvement is the identification of host gene segments that contribute to pathogen resistance. An antiviral factor, proteinaceous in nature, was detected in tobacco plants with the N resistance gene to TMV (Sela, 1981; Gera and Loebenstein, 1983). This factor was found to inhibit cucumber mosaic virus and potato virus X, in addition to tobacco mosaic virus. A nonspecific mechanism could be useful when detected in cultivated or wild species. The development of cDNA probes to detect such a gene segment(s) may open new avenues for the control of plant viruses.

Development of Diagnostic Kits

Evaluating the natural distribution and economic importance of plant pathogens and beneficial microorganisms would be most useful if carried out within national laboratory systems. However, several national laboratories in the WANA region are not adequately equipped and staffed so as to develop the materials needed for precise diagnosis. No laboratory in the region for example, is prepared at present to produce monoclonal antibodies.

Providing diagnostic kits to such laboratories permits workers to carry out the needed tests in their own laboratories and with the existing facilities. Diagnostic kits for specific rhizobia strains, BYDV, legume luteoviruses and other seed-borne pathogens listed in Table 1 will be valuable to ICARDA and national research programs. ICARDA can play a role in providing such kits to labs in its region, and in training in the use of such kits.

The quality of diagnostic kits is important when used in laboratories where they lack facilities to quantify reactions. Simple procedures which depend on visual assessment are most useful. Antibody preparations that produce very little or no background reaction are appropriate for survey purposes where an ELISA reader is not available. With no background reactions, visual evaluation is accurate and practical. Monoclonal antibodies could have an advantage over polyclonals, since they normally have a lower tendency to produce nonspecific reactions.

Summary

Immunodiagnostic techniques have become an important component in viral and microbiological research at ICARDA, where research efforts focus on improvement of cereals and legumes for food and forage. Polyclonal antisera have been used effectively for detection and field surveys for a number of plant viruses, but more

recently barley yellow dwarf virus (BYDV) monoclonals have proved to be useful in identification of the PAV, RPV and MAV types. The narrow specificity of some monoclonals is useful in detecting variability, but makes them inappropriate for field surveys where some isolates could go undetected. Specificity of PAV monoclonals originating from different laboratories was found to differ.

The autecological capability of polyclonal techniques, particularly, fluorescent antibody and ELISA, allows investigations of critical areas of *Rhizobium* behaviour in soil and especially *Rhizobium*-plant interactions in the rhizosphere.

Future applications of immunodiagnostic and DNA hybridization at ICARDA will include cereal and food legume luteoviruses and seed-borne pathogens. ICARDA will work to develop and provide kits for use by national program scientists in the WANA region, and will train individuals in the proper use of the techniques.

References

Ashby, J.W. and Huttinga, H. 1979. Purification and some properties of pea leafroll virus. Netherlands J. Pl. Pathol. 8: 113-123.

Benhamou, N., Parent, J.G., Garzon, S., Asselin, A., Oullette, G.B. and Joly, J.R. 1987. Use of monoclonal antibody against poly (1): poly (c) for detecting mycoviruses and potential applications to potato spindle tuber viroid and animal reoviruses. Can. J. Plant Pathol 9: 106-114.

Brochwell, J., Schwinghammer, E.A. and Gault, R.R. 1977. Ecological Studies of root nodule bacteria introduced into field environments. Soil Biol. Biochem 9: 19-24.

Casano, F.J., Hung, S.Y. and Wells, J.M. 1987. Differentiation of some pathovars of *Pseudomonas syringae* with monoclonal antibodies. EPPO Bulletin 17: 173-176.

De Pace, C., Delre, V. and Porceddu, E. 1987. The use of cloned hybridization probes to test barley yellow dwarf virus infection in durum wheat germplasm. In Abstracts, CIMMYT, Workshop on Barley Yellow Dwarf, July 6-11, 1987, Udine, Italy.

Duffus, J.E. 1979. Legume yellows virus, a new persistent aphid-transmitted virus of legumes in California. Phytopathology 69:217-221.

Dughri, M.H., and Bottomley, P.J. 1983. Complementary methodologies to delineate composition of *Rhizobium trifolii* populations in root nodules. Soil Sci. Soc. Am. J. 47: 939-945.

Dunez, J. 1987. Perspective in the control of plant viruses. pp 297-324 *in* Innovative Approaches to Plant Disease Control, I. Chet (editor). J. Wiley & Sons, New York, 372 pp.

Forster, A.C., McInnes, J.L., Skingle, D.C., and Symons, R. 1985. Non-radioactive hybridization probes prepared by the chemical labeling of DNA and RNA with a novel reagent, photobiotin. Nucleic Acids Research 13: 745-761.

Fuhrmann, J. and Wollum II, A.G. 1985. Simplified enzyme-linked immunosorbent assay for routine identification of *Rhizobium meliloti* antigens. Appl. Environ. Microbiol. 49: 1010-1013.

Gera, A. and Loebenstein, G. 1983. Further studies of an inhibitor of virus replication from tobacco mosaic virus-infected protoplasts of a local lesion-responding tobacco cultivar, Phytopathology 73: 111-115.

Hammond, J., Lister, R.M. and Foster, J.E. 1983. Purification, identity and some properties of an isolate of barley yellow dwarf virus from Indiana. J. Gen. Virol. 64: 667-676.

Hampton, R.O. 1983. Pea leaf roll in northwestern U.S. pea seed production areas. Plant Disease 67: 1306-1310.

Hull, R. 1984. Rapid diagnosis of plant virus infections by spot hybridization. Trends Biotechnol. 2: 88-91.

Iannelli, D., Capparelli, R., Marizano, F. Scala, F. and Novallao, C. 1983. Production of hybridomas secreting monoclonal antibodies to the Genus *Fusarium*. Mycotaxon 18: 523-532.

Jedlinski, H., Rochow, W.F. and Brown, C.M. 1977. Tolerance to barley yellow dwarf virus in oats. Phytopathology 67: 1408-1411.

Jordan, R.L. and Hammond, J. 1987. Epitope analysis of potyviruses coat proteins using monoclonal antibodies. Abstracts. 7th International Congress of Virology, August 9-14, 1987. Edmonton, Alberta, Canada.

Leary, J.J., Brigate, D.J. and Ward, D.C. 1983. Rapid and sensitive colorimentric method for visualizing biotin-labelled DNA probes hybridized to DNA-immobilized on nitrocellulose: bio blots. Proceedings of the National Academy of Sciences, USA 80: 4045-4049.

Lorens, G.F., Falk, B.W. and Qualset, C.O. 1987. PAV concentration differences among field resistant and susceptible cultivars measured by northern dot blot analysis with the RPA8 cDNA clone: *In* Abstracts, CIMMYT Workshop on Barley Yellow Dwarf, July 6-11, 1987, Undine, Italy.

Macquaire, G., Monsion, M., Mouches, C., Candresse, T. and Dunez, J. 1984. Spot hybridization: Application to viroid identification. Ann. Virol. (Inst. Pasteur) 135E: 219-230.

Makkouk, K.M., Azzam, I.I., Skaf, J.S., El-Yamani, M., Cherif, C. and Zouba, A. 1987. Situation review of barley yellow dwarf virus in West Asia and North Africa. Abstracts, CIMMYT Workshop on Barley Yellow Dwarf, July 6-11, 1987, Udine, Italy.

Makkouk, K.M., Bos, L., Azzam, O.I., Koumari, S. and Rizkallah, A. 1988. Survey of viruses affecting faba bean in six Arab Countries. Arab J. Plant Prot. 6: (in press)

Mitchell, L.A. 1986. Derivation of *Sirococcus strobilinus*-specific monoclonal antibodies. Can. J. for Res. 16: 939-944.

Mitchell, L.A. and Sutherland, J.R. 1986. Detection of seed-borne *Sirococcus strobilinus* with monoclonal antibodies in an enzyme-linked immunosorbent assay (ELISA). Can. J. for Res. 16: 945-948.

Morris, T.J., Dodds, J.A., Hillman, B., Jordan, R.L., Lonnel, S.A. and Tamaki, S.J. 1983. Viral specific dsRNA. Diagnostic value for plant virus diseases identification. Plant Mol. Biol. Rep. 1: 27-30.

Olsen, P.E. and Rice, W.A. 1984. Minimal antigenic characterization of *Rhizobium meliloti* strains by indirect ELISA. Can. J. Microbiol. 30: 1093-1099.

Owens, R.A. and Diener, T.O. 1981. Sensitive and rapid diagnosis of potato spindle tuber viroid disease by nucleic acid hybridization. Science 213: 670-671.

Probhakar, B.S., Haspel, M.V. and Nokkins, A.L. 1984. Monoclonal antibody techniques applied to viruses. In Methods in Virology Vol. VII (K. Maramorosh and H. Koprowski, eds.) Academic Press, New York, pp 1-18.

Sela, I. 1981. Interferon-like factor from virus-infected plants. Perspect. Virol. 11: 129-139.

Sela, I. 1987. Genetic traits to be manipulated in plants for virus disease resistance, pp 325-335. *In* Innovation Approaches to Plant Diseases Control. I. Chet (editor). John Wiley Sons, New York, 372 pp.

Skaria, M., Lister, R.M., Foster, J.E. and Shaner, G. 1985. Virus content as index of symptomatic resistance to barley yellow dwarf virus in cereals. Phytopathology 75: 212-216.

Tchen, P., Fuchs, R.P.P., Sage, E. and Leng, M. 1984. Chemically modified nucleic acids as immunodetectable probes in hybridization experiments. PNAS (USA) 81: 3466-3470.

Vincent, J.M. 1982. Serology pp. 235-273 *In* Nitrogen Fixation Vol. 2, J.W. Broughton (editor). Oxford University Press, New York.

Waterhouse, P.M., Miller, W.A., Cheng, Z. and Gerlach, W.L. 1987. General and serotype-specific nucleic acid probes for the detection of barley yellow dwarf virus. *In Abstract*, CIMMYT Workshop on Barley Yellow Dwarf, July 6-11, 1987, Udine, Italy.

Wright, S.F., J.G. Foster and Bennett, O.I. 1986. Production and use of monoclonal antibodies for identification of strains of *R. trifolii*. Appl. Environ. Microbiol. 52: 119-123.

One Company's Attempt to Commercialize an Agricultural Biotechnology Technology

Dr. Peter S. Carlson, Vice President
Crop Genetics International
Hanover, MD 21076

It's a pleasure to be here. We've all been together for several days now, and we all have heard enough speeches about the world as it should be. I'd like to invite you to see the world as it is. I will tell you my story. Because it is my story, I know it best, and that story is probably typical of a number of attempts to commercialize the fruits of agricultural biotechnology. I started out at Crop Genetics about seven years ago asking the rude, typically American question of: "If I'm so smart, why aren't I rich?" Unfortunately, I don't have the answer to that question today. The jury is still out. Along the way, I have learned some things: learned some things about science, learned some things about the world, and learned some things about bringing technology to the market.

I'll give you the take-home one-liner first. What I've learned is, you have to bloom where you're planted, an axiom my mother imparted to me many years ago. I didn't really believe or understand it back then. You've got to take the world as it is. And as a businessman, that's a critical realization. Once you've accepted the world as it is, then you can begin to attempt to change it.

I started with beginning Crop Genetics seven years ago, thinking that plant tissue culture was going to be the biotechnology engine that would change the world. Crop Genetics' first business plan was very interesting. We had looked around at tissue culture and thought: where is the value, what can tissue culture do that is unique, where can we really create value and something that didn't exist before? It was obvious that there were a lot of individuals out there in the plant genetics business: the Pioneers, the Dekalbs, the NKs, many universities, and a lot of corporations in the ag/chem business. We didn't want to be either of those: we didn't have an existing market and we were a small company. So tissue culture in bananas and sugarcane seemed reasonable. Our first business plan focused on changing bananas to be resistant to black sigatoka and producing disease-free sugarcane.

We have dropped bananas because it was very difficult to find a place or customer to sell the product. It wasn't something that a private company could do. In sugarcane, we are now a two million dollar a year business in Louisiana and it's very telling. Tissue culture is only a fraction of our total costs. We make plantlets in

our laboratory, send them to Louisiana, grow them up at our quarantine farms in Louisiana, cut the stalks and sell to the farmer what the farmer now produces for himself, that is, stalks of cane for seed. The difference is that ours don't contain diseases. His do. The consequence is a twenty to twenty-five percent increase in yield over the three-year life cycle of the crop. Now, we also learned that besides creating that twenty to twenty-five percent value, we had to have something called repeat sales. In Pioneer's case and in Dekalb's case with corn hybridization is the repeat sales mechanism, because the hybrid reverts back to the lower yielding inbreds and yield decreases. The farmer can't save seed and use bin run material. In our case, sugarcane, that repeat sales mechanism is the re-introduction of diseases. Systemic diseases drive the farmer back to buy from us once again.

That's all very interesting, but my constituency is more on Wall Street than it is in the farmer's field. Let me tell you what I have to do to bloom where I am planted; because my constituency is Wall Street, the venture capitalists and the SEC and not the CGIAR and A.I.D. Let me give you a presentation that is designed for businessmen. This is made to give them the lift of the driving dream, to allow us to commercialize technology. Let's go through that story slide so that you can realize the kinds of constraints that we, as a company, have to meet. After having done that, I hope you'll have a better appreciation of the kinds of things we can do and can't do, given the constraints within which we live. Perhaps we, collectively, can generate some kind of mechanism where the concerns and needs of the private sector can interface with the concerns of moving biotechnology out to the worldwide market. We all know that we can create tremendous value with biotechnology. I feel that there are only small numbers of subsections of that technology that will be opportunities for the private sector. The majority of plant biotechnology will, in the end, I believe, find it's home in the public sector institutions.

We biotechnologists got our message out to the world about fifteen years ago and we said, "There's a revolution coming". Well, where is it? Besides, people don't like revolutions. In revolutions, when the Bolsheviks took over Moscow, and when Mao came back into Beijing, people got killed. Generally, society doesn't like to hear about revolutions. When we talk about revolutions, we create all kinds of scary scenarios that provoke activists and then regulators into action.

In terms of technology making the difference, here are the components that are really important to be able to create a business in the ag/biotech sector. I think there are four. To make significant ad-

vances in major crops, you need a breakthrough technology. That's what biotechnology is.

Secondly, you need a high margin product. In the seed industry, outside of corn, there are not many high margin products. The seed industry is not generally perceived as being a good business, unless you have a repeat sales mechanism like hybridization. Thirdly, you need large markets. Because the development costs of so many biotechnology products is very expensive, you have to have markets that once the products are out there, you can generate a cash flow, which will be able to support the cost of research and development and marketing for those particular products.

And lastly, you need repeat sales. You know, when we were in tissue culture, we went to see Tom Urban at Pioneer Hybrid. Tom said, "You know, you've got a great technology. Tissue culture is just wonderful. People love to see little plants in test tubes. But you know, that's all you've got. You've got a technology. You don't have a business. To have a real business, you have to sell the same product to the same customer year after year after year." I think that law, that iron law, is the dividing line between the activities of the private sector and the public sector in ag/biotech.

Well, what does all this mean? This means we focus on high margins and large markets and repeat sales, that for the time being biotechnology will be focused primarily on first world activities. This is where the technology is going to break, this is where the financial managers of the world can see the existing markets and say okay, you go after that market and you can get it, this is what it means. If an MBA or a businessman cannot quantify the potential results of success even when it occurs, you're never going to get any money to support that particular project. You can't spend money and keep your job at the same time by focusing on markets that don't now exist.

Our answer to these four questions is InCide. InCide is a new delivery system for biological crop protection. Generally, at Crop Genetics, we see there are three ways to protect crops. First of all, you can do plant breeding and generate new genotypes. I think that molecular biology and the insertion of novel genes is a subset of plant breeding. You can modify the plant genome. Secondly, you can spray something on the plant. You can spray on a chemical or biological, and the consequence is you can protect the plant from a fungus or an insect or whatever.

The third way to protect the plant is something that we've invented called a plant vaccination. That is, you generate a microbe that can

be applied to the seed, at the seed conditioning plant. When the seed is bagged, the microbe stays there. Then the seed is planted, a simultaneous germination of the seed and the microbe breaking dormancy. The microbe lives within the plant. It's an endophyte. It lives within the plant for the life of the plant, doing whatever it's supposed to do, making an insecticide or a fungicide, but it is not seed transmitted. Because it is not seed transmitted, it's good for public concern: it doesn't spread. It's even better for business because it means repeat sales.

We see the InCide technology having applications in three major areas in the crop protection market: insecticides, fungicides, and plant growth enhancers. Crop Genetics has a collection of about a 1000 endophytes, able to colonize corn, cotton, sorghum, soy, wheat and rice. We are a biological delivery system company. We have a delivery system, endophytes, that can be modified by the tools of genetic engineering to do something positive in all of these crops. We are doing plant genetics by proxy, not modifying plants, but modifying the characters of plants by getting microbes to do something they wouldn't ordinarily do.

How does it work? We use two raw materials and one tool. The tool is recombinant DNA. Because we are using recombinant DNA, we're at the center of the public policy controversy. We take two raw materials. First a gene, that encodes for some interesting agronomic function, be it an insecticide or a fungicide or a plant growth enhancer. This raw material came from the pharmaceutical industry. That is, any particular antibiotic or antifungal that has been found over the years by the pharmaceutical industry, can now be inserted into an endophyte that knows how to express that particular characteristic and then used for crop protection.

The second raw material is an endophyte. An endophyte is a microbe that lives in the plant without causing a consequence to that plant. Now, endophytes have been around for along time, and we in tissue culture knew they existed, because whenever we made explants, we had a lot of contamination. Microbes do exist in plants, the plant contains a rather active microflora, even though you can't see it in terms of plant disease or plant yield, they still do exist. Endophytes weren't of any use until we decided to make them the basis of our delivery system. Those are the two raw materials. When we make the recombinant, it is an endophyte that will express the particular agricultural chemical function and that is then inserted into the plant.

Endophytes are, by and large, systemic. That is, they occur in all the tissues of the plant. You can only insert in a small amount of

endophytes in seeds because the final scale-up occurs in the farmer's field during the growing season. Only very small amounts of the endophyte are needed to fully protect the plant. The consequence of this is that InCide will not be a curative treatment. Since the scale-up occurs in the field, we have a lag period. InCide will be sold as a crop preventative rather than a curative. That's a function of the biology of endophytes. We have a plant that has within its tissues endophytes, that are doing something interesting, so that the target pest that has the poor judgement to attack that particular plant is met with a biological challenge, be it a protein or a low molecular weight secondary compound, able to counter its attack on the plant. Consequently, the crop is protected.

We have identified endophytes able to colonize all major crop species. As a small company, we have focused in on corn, because corn is the largest market, in the United States, and corn has got some problems that our technology can solve. We are focused on North America because that's where we're planted. Corn is a crop where there are problems that cannot now be solved by either plant breeding or by chemical sprays. Our corn endophyte, which is a microbe called *Clavibacter xyli* subspecies *cynodontis* (Cxc) exists in the corn xylem, and in the subtending air spaces. It exists throughout the plant in fairly high concentrations of about 108 Cxc per gram of plant fresh weight. When translated back, that's somewhere about 109 or a little higher, per milliliter of xylem sap.

This endophyte is our delivery system, so that a plant that contains it then will have millions of little bacterial working factories to protect the plant. InCide products can be applied to plants either as a spray, or in the seed. We think, as a practical matter, that seed application is much more attractive. If microbes are introduced into the seed, the microbe is contained in the seed and when a seed germinates, InCide is contained within the xylem of the plants.

We can deliver Cxc into the seed and get colonization of seeds by using a couple different techniques. We can use solvent containing the InCide bacteria and vacuum or pressure. For example, we can take Cxc, put seeds in that particular InCide-containing solution, and pull a vacuum. The seed contains a number of small air spaces that develop during dry-down. Those air spaces begin to expand under vacuum, the air bubbles off, so that when the vacuum is released the InCide bacteria is pulled into the seed and resides in a stable fashion within the seed. A simple technique, but it works.

What's the InCide market? The InCide market is the fifteen billion dollar a year chemical crop protection market. Billion is a better word than million on Wall Street, and that's where we're planted.

The ag/chem industry and *Bacillus thuringiensis* (Bt) began about the same time after World War II. Bt was used in France in 1939. So was DDT. Generally Bt and biologicals lost the race. They only constitute about 50 million dollars of the spray crop protection market. Chemistry has won the race because it works and it's inexpensive. Bt and other biologicals have lost the race because they are not as efficacious as chemicals and they are more expensive. Its efficacy and cost are the real issues. One of the major problems with chemicals and Bt biopesticides is that they are both externally applied. In spray-on chemicals, only a very small portion, less than one percent, of the chemicals actually reach the target pest. That means more than 99 percent go elsewhere in the environment. That creates problems over application of residues, and of groundwater contamination, both which are of public concern. Biopesticides like Bt, are also applied externally and they are very sensitive to environmental degradation. They are sensitive to UV from the sun, rain, to wind, so that when you spray microbes on plants, they are degraded more readily in the environment that most chemicals. External application is inefficient when chemicals and biologicals are externally applied.

If external delivery is the problem, what is the solution? The solution is delivering biopesticides inside the plant, and that's really what InCide is all about. It's taking the value that now exists in the ag/chem industry and moving that back into the seed industry.

Generally, the seed market is worldwide 24 billion dollars, and ag/chem is 15 billion dollars. Of that 15 billion ag/chem market, about a one-quarter billion is chemical seed treatment, the fungicides and the insecticides that are put on seeds during the conditioning process. InCide is going to move more value from the ag/chem industry to the seed market because it is something that the seed conditioning can put on during that process.

Follow-on products with the InCide technology include insecticides for corn, cotton, rice and soy. We chose corn, cotton, rice and soy because they are major American and worldwide crops. We also chose insecticides because the technology fits the bic-pesticidal genes available. Those of us who are geneticists never like to say that our technology is immature. Geneticists can move single genes around, but when it comes to moving pathways from microbe to microbe, that's something very difficult. Fungicides are primarily low molecular weight excreted products that take a whole pathway to produce. You start from basic metabolities, go through a couple of different steps, and end up with a fungicidal molecule. That is too difficult for the current tools of recombinant DNA. However, insecticides from Bt, the delta endotoxin protein in particular, are

appropriate for the tools of recombinant DNA. Single genes work so we focused on single genes, and chose several different market opportunities.

Our first product target is the European corn borer. In the U.S. Midwest, the corn borer, *Ostrina nubialis*, goes through two generations. Plant breeders have been able to find some resistance to the first generation in corn because feeding is primarily on the leaf and in the whorl. Chemicals can be used on the first generation borer, because the tractor can still get in the field to spray. The first generation borer really is not our target. Our target is the second generation borer. Our target is the second generation borer because plant breeders don't have resistance to second generation borers, and the borer gets into the actual corn stem and eats its way up and down in the internodal areas, so that applied chemicals never actually can get to the borer, because the borer is in the plant. During the second generation it's late enough in the season, so you have to use contact rather than systemic insecticides.

Control of the second generation borer is a problem for which there is no current solution. As a small company, that's one more thing we like. We'd like to be nobody's competitor. We choose to aim the InCide technology at an unsolved problem. Control then, can be gotten not with pounds of Bt or a pound or two of chemical, but with nanograms per acre. We can apply very low amounts. You think about putting in 106 microbes per seed and there being somewhere about 22,000 seeds per acre. When you do the calculations, we only need nanograms of the product to have borer control for the acre. The product is valuable, because the scale-up occurs in the farmer's field after point of sale. What are the consequence of that? InCide can replace 30 million pounds of active chemical ingredient, of chemicals sprayed into the environment by about 30 pounds of InCide. It's a million to one advantage. We could take care of the whole corn market with about a 1,000 liter fermentor running in our facility. The InCide cost structure is very different than sprays, and the opportunity is different too.

The European corn borer is a problem throughout the U.S. and Europe. In the U.S., it causes about 400 million dollars a year of damage to the corn crop, even after 50 million dollars is spent for chemicals to control it. The majority of that damage, about 80 percent, is second generation borer damage. So, the European corn borer is an unresolved problem. That's also true in Europe. True to its name, the European corn borer was an immigrant like most of us to the U.S. It came over from Europe about 80 years ago, before my family arrived, and it is also a major pest on corn in Europe. It is

the major French corn insect pest. There, it causes about 50 million dollars of damage, even after 25 million dollars of insecticides are used for its control. So it's a worldwide opportunity for InCide.

Now, what about follow-on InCide products once we control the European corn borer? Are there other opportunities in corn? And the answer is yes, there are other insects for which Bt genes are available. There is the cutworm, and also the earworm, which are lepidopterous insects that attack corn for which Bt genes are available. Of course, in the long run, the corn rootworm, a coleopterian, is a target. So there is an opportunity to generate more than one product.

What feature of InCide is attractive in the U.S.? The problem we have in the first world is, the perception that there is too much food already. The first world doesn't realize that the majority of the world doesn't have too much food and is very parochial. Consequently we have a very hard time making the argument that we're going to increase food production. We have an easier time making the argument that we can lower costs. Surely that ought to be the argument, that is, more food is better, to make outside of the developed nations. The primary argument we are forced to make, because we're blooming where we are planted, is the environmental safety issue. InCide can aid the environment and improve corn at the same time. Now this is a real issue.

Investors in small companies want to know how you're going to sell this technology. We're going to sell it to farmers through the seed companies. What does the farmer get? What's the get for the give when the farmer buys our product? In Iowa, average yield last year was 130 bushels an acre. The corn borer will cause about 20 percent damage in areas of the pest damage. When you look at the consequences of that, we think that we can raise the yield of corn in Iowa from 130 to 145 bushels just by European corn borer control. Revenue, as consequence of that, at the current price of $2 a bushel would be increased by about $25. Costs would be decreased, because we can sell InCide as protective, applied in the seed plant for a lower cost than a farmer would have to incur spray-on chemicals, both the chemical and the spray costs, in treating the European corn borer. That's what you make for the farmer. It's lower costs and higher revenue. Well, if you're going to sell to the seed company, you have to have a get for the give there too. How does that work? The seed company is going to have higher revenues. As an example, a seed company has one percent of the U.S. market, about 200,000 units, each a bushel. Today, just about $10 an acre per unit and then with insight, since you're adding value to the seed, you can ask the farmer to pay you more

for the seed. That's the big discussion. And the question is, how much can you ask? Assuming a profit, we're going to double the profit of the corn seed conditioning companies by this particular innovation. So the seed company gets something, the farmer gets something, we get something, and we all win.

Intellectual property is very important in the first world and I'm going to tell you small companies have different constraints compared to large companies. Large companies have product sales. They've got cash flow. They can afford their R&D research off that cash flow. When you're a small company, and you've got minimal product sales, you're financing research off money from stock sales. It's a very different kettle of fish. Generally, small companies are vulnerable when it comes to intellectual property, so patents are important. We have patented the InCide system. We've patented the general idea of delivery of agricultural crop protectants through endophytes. No one else did that before we got here. We can also patent the components of InCide for use in that delivery system. The endophyte is patented for that use. Bt genes for that use can be patented. We can also patent the individual products against the corn borer. And we can also patent the inoculation methods, since no one has developed, prior to this time, a method putting microbes into the seed. Those levels of patents protect us allow ample time to do the R&D, to do the marketing, to do the sales, to recoup the costs that we incur along the way.

There it is; technology makes a difference at Crop Genetics International. What I hope we agree upon after this talk is that technology is only one of a number of components that makes a difference. You've got to have marketing. You've got to have intellectual property. You've got to have repeat sales. Technology in a vacuum is nice, but technology in a vacuum is not a business. That is a critical component where we are planted.

Let me just say a few words about cooperation with small companies and international agricultural institutes. I hope I have given you an idea of the kinds of constraints that we face; I know that you also face constraints in your own institutions. You have to live by the laws of the institution, the laws of the government, and things are complex. However, I would like to stress that small companies are very flexible. If there is one thing that is characteristic of small companies, it's a lack of bureaucracy, it's a lack of business dogma. There is not one way to do things, and the *flexibility* in trying to generate a mechanism to allow us to work together, I feel, really is the key word. In closing, I would like to say that we have to bloom where we're planted. We have to take the world as it ex-

ists, because that's just the way life is, but that's no reason to stay there. And by understanding each other's institutions, maybe we can take the world to a place where it ought be, even though it isn't there now.

A New Era of Government-Industry Cooperation in Research and Development

William Tallent
Agricultural Research Service
U.S. Department of Agriculture
Washington, D.C.

The new era in industry- government cooperation in research and development is fueled primarily by the new law that was passed in 1986. It is called the Federal Technology Transfer Act of 1986 or Public Law 99-502. I want to talk a little bit about how and why it came about, what its impact has been, and what are some of the implications of it vis-à-vis the subject and focus of your conference here.

Basically, PL 99-502 authorizes closer working ties between government research laboratories and industry in the area of research and development. It enables us to do in government laboratories what universities have been doing for some time—develop cooperative agreements, joint research projects, consortia, and those sorts of things. I'll come back to some of the specifics in a minute, but let's talk first about the background or evolution of this new law. It really started back in the late 70s with a study led by a man named Jordan Baruch, who was an Assistant Secretary of Commerce in the Carter Administration. That led to something called the Stevenson-Wydler Act of 1980, which talked about cooperation between government research organizations and the private sector.

But the Stevenson-Wydler Act, quite frankly, was mainly a philosophical expression, you know this is God and motherhood, this is good, so why don't you do it. It didn't have many specifics for implementation. Also as a result of the Baruch Study, was something that did have a bit more practical implications, namely, a revision of the patent law, which enabled government research laboratories to give exclusive licenses to developments in those laboratories. The patent law revision also enabled us, when we fund research at a university or a small business under contract, to give title to the inventions to that organization. Now that was specific for nonprofits, mainly universities and small businesses, but since then, there was first an executive order and then another change in the law, which said you can do this with all businesses.

Then, in the transition period between the Carter and the Reagan Administrations, there was a study led by David Packard of Hewlett-Packard, so it was called the Packard Study or the Packard

Report. You know, we in government heard all about Grace Report in those times, and we all were cringing at the terrible things that were going to happen as a result of that. The Packard Report, although it was fairly little noticed, had some impact and had some important things to say. One of these was that federal labs are here to stay. They have a constituency and are needed, but we need to use them one heck of a lot better. And a number of specific proposals were made, some of which have been subsequently implemented. My point is that this new law (PL 99-502) and this new era have evolved through both Democratic and Republican administrations. Public Law 99-502 passed with an overwhelming majority in both houses of Congress, and had Democrat and Republican support. As we approach another election, that's important, because what I perceive is no matter who wins, this impetus, this new era, is going to continue, because I have seen statements of support for this kind of cooperation from both sides.

Why did all this come about? First of all, there was a perception that research and development results out of federal labs were not very well used. This, I hasten to say, may not be true, and I hope it is not true for agriculture. We have a tradition of our extension service and a tradition of working closely with agribusiness firms, but when you look at the big DOD and DOE labs, any one of them is as big as the Agriculture Research Service altogether. And so when you start looking at what's come out of them, in terms of impact in the economy and the so-called spinoff, the technology that is there that could be used, the perception is, and probably rightfully so, that we are not getting near the bang for the buck that we should get. Even for ARS, I can cite examples were it has taken five, ten, even fifteen years for a piece of technology to come from the laboratory out to commercialization, and when it did get commercialized, it made quite an impact.

But that time span is just unacceptable, given today's needs, urgent needs, for use of that technology that comes out of government laboratories to solve our economic, our human health and welfare, and our environmental problems. And the situation would get worse if nothing was done. There is, has been for maybe ten years, a push for government labs to move back toward fundamental and basic research; not get into applied, leave that to business. At the same time, under fierce economic competition, industry has been moving the other way, and there have been some reports that pointed out that there is a gap developing. This law (PL 99-502) then moves the two sectors back together, provides an overlap, provides for government to work side-by-side and bridge that transition. And that's really what it is about, trying to bridge that transition and shorten the time between discovery and application.

Finally, the Japanese model was a big factor. If we learn anything from the Japanese, we ought to learn that government and industry need to be partners rather than adversaries.

Now, some specifics of the new Federal Technology Transfer Act. It says that federal labs can accept funds, personnel, services, property, information, etc., from industry. We can provide the same thing to industry, except for funds. And the reason for that exception is that if we could provide funds, this law (PL 99-502) would become a procurement act and then it becomes burdened with all the bureaucratic process that tries to be fail-safe to make sure federal funds are used properly. So by leaving funds out, the opportunity is there a[illegible]he whole tenor of the law is that the agreements need [illegible] flexible, simple, and rapid. We need to keep the bureaucracy out of this and make it a simple, straightforward process. Now there are few people in this room who have been through this with us, and I don't know whether they will agree with me that it is simple and rapid and flexible, but we try.

Notice personnel can go both ways under PL 99-502. One of the things we need to do more of is scientific exchange. Put scientists from government labs into industry labs and vice versa. We have done a little bit of that, but there is an opportunity to do a lot more, and I think the benefits would be great. Another thing PL 99-502 specifically provides for is that a federal scientist inventor can move in and help industry in any number of ways to begin to commercialize his or her invention. That's either while he or she is still federally employed or after retirement. And after retirement is an important point—we've got a couple of scientists now who are preparing to retire. There are firms wanting to pick up their inventions and after retirement they can go right into industry. Now the personnel people used to, and in fact still do, shudder at that kind of thing and say that is a conflict of interest. But it is not. Scientists can and we should encourage them to go in and help industry develop their inventions. And those of you in industry who have been involved in this know that you can write or talk until you are blue in the face, but there is no substitute for being right there helping to commercialize or develop an invention. You can't put everything in writing. There are little things that just don't get said, that are vital to getting to scale-up and development and making something work on a production scale, that's been made to work in a test tube in a laboratory.

Another very important part of the new law is that federal research and development agencies must now report activities under PL 99-502 annually as part of their budget process (now if anything makes us sit up and take notice in the federal bureaucracy, it's when you say "budget." That is, as part of the process, when you

submit your new budget, you have to say what you have done in the past year to implement PL 99-502. Before, under the Stevenson-Wydler Act, we had to make a report every two years. We made it to the Department of Commerce. These are nice, fat documents. I bet you a dollar that there are not a half dozen people anywhere who have ever read them. But when you make it part of your budget submission, it gets attention and we give more attention preparing it, too. In that regard, we're going up to the House next Tuesday. My boss is to testify on our budget. We have our report in and I might just correct the abstract. It says that we have completed seven of these agreements and have thirty-five in process. That was true at the time it was written. We now we have twenty completed and thirty more in process. Among the completed ones incidentally is one with Dr. Carlson's company.

The Federal Technology Transfer Act of 1986 was passed by Congress and signed into law by the President in October 1986. We were looking at it, studying it, scratching our heads about it, but not really doing very much—the whole government research and development sector. So in April, the President issued Executive Order 12591. It was April 10, 1987, almost exactly a year ago. In a sense, that executive order said, "Come on you folks in the government research and development community, get on with it!" The executive order goes a little bit farther and establishes something called "technology share centers." This addition is important because it brings the university sector into the act and talks about university-government-industry consortia or sharing arrangements. And under these, we can put in federal money on a one-for-three matching basis. That is, every federal dollar has to be matched by three dollars from the private sector and the state and local organizations. State and local involvement is important because there are many efforts to utilize these kinds of arrangements to stimulate local economy. So we have several of these under way.

Typical provisions of our agreements under PL 99-502 are as follows. We speak of *patents* and we say fairly simply, I think, if under the cooperation something is invented by an Agricultural Research Service (ARS) scientist, ARS takes title to the invention; if it is joint, we have joint title; if it is conceived by the industry, it's their patent. If those patents belong to ARS or are joint, we give the cooperator right of first refusal to an exclusive license. The National Technical Information Service (NTIS), a division of the Department of Commerce, handles the licensing of ARS patents on a reimbursable basis for the cost of doing that job for us, and there are some advantages to this arrangement. It puts them in the position of negotiating the fees and royalties with the cooperator and not us, and so that kind of negotiation, which can get a little sticky, does not get in the way of the cooperation.

Confidentiality. This is a little bit new for government research. We are usually wide open, but when we have one of these agreements, we will keep results confidential long enough to protect them by patents.

Publication. We'll give the industrial cooperator 60-90 days to review a manuscript. We will seriously consider the suggestions that the industrial cooperator may have for changes in that manuscript. We stop a little bit short of giving them an absolute veto power of what goes into manuscript. And I think that's also true of the university-industry cooperation.

Each side has responsibility for getting its own *regulatory clearance*. Now that's an important point. We keep arm's length in the Department of Agriculture and other federal departments between the research component and the regulatory component. There are some particularly small businesses that may come in and may think, "Well gee, I'm working with the Department of Agriculture. I don't have any problems with regulation. They'll take care of that." So we take pains to point out in the initial agreement, we won't. They still have to deal with the parts you are going to do in the cooperation with the regulatory agencies, be it the Animal and Plant Health Inspection Service (APHIS) or the Food Safety Inspection Service (FSIS), and so forth. The part that we do, e.g., the biotechnology experiments that we do, we will get our own clearance and put them through our biosafety committee and so forth ourselves. But if the industry is going to do, say field tests or something that is a scale-up of the research, they have to get that clearance.

Our program to implement PL 99-502 is described briefly in a brochure entitled *Cooperative R&D Agreements between Industry and ARS*. Copies are available from my office.

Now, let's talk a little bit about the international aspects. That's what your conference here is all about. The law (PL 99-502) is not silent on the international aspects. As a matter of fact, maybe it's a little bit defensive in that regard. There is a provision that says that "The laboratory director, . . ." (I am reading from a piece of the Federal Law 99-502) ". . . in deciding what cooperative research and development agreements to enter into, shall . . .," and it lists a couple of things, and one of them is ". . . give preference to business units located in the United States." There is a careful distinction in language there. Not U.S. companies, but business units located in the United States. If a CIBA-GEIGY has a United States component, that is perfectly fine. We can cooperate with them. ". . . which agree that products embodying inventions made under the cooperative research and development agreement or pro-

duced through use of such inventions will be manufactured substantially in the United States and, in case of any foreign company or government, as appropriate, take into consideration whether or not such foreign government permits United States' agencies or organizations or other persons to enter into cooperative research and development agreements and licensing agreements." In other words, there needs to be some kind of reciprocity. In this regard, the executive order says, and I will just read an excerpt here, "The head of each executive department and agency, when negotiating or entering into cooperative research and development agreements . . ." (that's the name for the agreements under this new law) ". . . and licensing arrangements with foreign persons or industrial organizations (where these entities are directly or indirectly controlled by a foreign company or government) shall in consultation with the United States Trade Representative, give appropriate consideration to whether such foreign companies or governments permit and encourage United States agencies, organizations, or persons to enter into cooperative research and development agreements and licensing arrangements on a comparable basis." Reciprocity again. And also, and this is an important point, "to whether those foreign governments have policies to protect United States intellectual property rights." I might say that there is an interagency task force led by the Department of Commerce working to and yet develop processes for international cooperation under this PL 99-502 and Executive Order 12591 that will be faithful to these provisions.

Concerning the part about the intellectual property, I want to say just a bit more. Dr. N.C. Brady's letter to the conference presentors said that we should address future problems or needs in areas where collaboration in research, commercialization, or policy development would be desirable. I think this area of intellectual property protection is such an area with regard to policy development. It is a major agenda item—intellectual property protection—in ongoing General Agreement on Trades and Tariffs (GATT) negotiations. It is also a part of the new science and technology agreement between the United States and Japan for scientific and technical cooperation.

We also need to change the United States laws to provide that imported products produced in a foreign country under a process covered by a U.S. patent would constitute infringement, and this is particularly relevant to this conference because biotechnology is so process-oriented.

Thank you for giving me the opportunity to share these developments and views with you.

Conference Comments: Biotechnology and World Food Demand

Norman E. Borlaug
Distinguished Professor of International Agriculture
Texas A & M University
College Station, Texas

Fellow participants, permit me to take a few minutes from the conference schedule to interject my perspective on the role that biotechnology will likely play, or not play, in the developing nations toward increasing agricultural productivity in the next decade or two.

For the past two days, I have listened with fascination to the reports on research in biotechnology and genetic engineering that bears on the future improvement of agricultural animal and crop productivity. The discoveries in this new field of research are rapidly expanding the spectrum of applications to agricultural and biological sciences and will provide entrée to many new frontiers. The enthusiasm manifested by the participants verges on euphoria; it is infectious and inspiring.

I want to congratulate and thank USAID for having had the perspicacity for organizing this conference to further the interest in the new broad field of biotechnology. Moreover, I want to congratulate all of the participants whose informative and enthusiastic presentations will undoubtedly greatly accelerate research in this broad new field of biology.

Let us hope that in the not too distant future, the new basic knowledge now being reported will be linked to that of other disciplines of agricultural sciences thereby resulting in an improved technology, which when applied will produce more food. Lest we forget, biotechnology will not function in a vacuum devoid of other scientific disciplines. Its success or failure will largely be determined by how it is linked to other scientific disciplines, especially to conventional plant breeding and genetics.

Simultaneously, with developing new research and technology capable of increasing crop yield and production, we must learn how to more equitably distribute the food produced. That should be the ultimate aim for all who work in agricultural sciences.

Before I proceed further, I would point out that I do not like the term biotechnology as it is now commonly used in conferences and

in the literature; it is too all-inclusive, covering the broad sweep of biological investigation from tissue culture, cell culture, and protoplast culture to manipulation at the molecular level. This broad loose usage, contributes to confusion, not only for the general public, but for many scientists as well.

Before I proceed with my comments on the future potential role of biotechnology in food production in both the developing and developed nations, let me digress to comment on the excellent research proposal made by Dr. Richard Baldwin and the members of the IFAR Committee. Their proposal to form "Manhattan Project-" or "Moon Landing Project-" like interdisciplinary teams of agricultural-biological excellence to attack some of the basic problems that will limit food production in the long-term future is an excellent idea. It would appear to me that the individual projects must be selected with care and limited in number to be economically viable. I like their choice of the project to attempt to supply most of the nitrogen requirements for wheat (or maize) with symbiotic nitrogen fixation by the year 2005. I am not, however, very enthusiastic about the proposal to produce hybrid soybean varieties, because of the limitations in amounts of pollen produced by soybean flowers. Nor am I very enthusiastic about cassava varieties with high levels of Vitamin A, because there are many other potential sources of Vitamin A in the tropics. On the other hand, a project to develop a high-yielding corn hybrid with considerably improved photosynthetic efficiency, or greater drought resistance, would have major significance. There are many other basic worthwhile deserving problems that might be solved by such a "team of excellence" approach.

Now permit me to return to the world food production and hunger problem that we must confront during the next one or two decades. As I look at the magnitude and complexities of the world food problems at present, I feel obligated to emphasize that we should never forget that research well done in one field or discipline does not automatically produce more food, nor does adequate food production alone automatically eliminate world hunger and malnutrition. We only need to reflect on what has happened worldwide over the last 15 years to see how complex many of the interrelationships are in food and fiber production and distribution. We have had a serious cereal glut in food-exporting countries since about 1983 or 1984 resulting in depressed agricultural prices. As a result, many farmers in developed food-exporting nations who were deeply indebted have gone broke. We should also remember that at the same time there were gluts in the food-exporting developed nations, there was hunger and famine in Africa. This is a consequence of inadequate food distribution. Lack of foreign exchange

(purchasing power) at the national level in many food-deficit African countries and lack of purchasing power at the family level within these nations is caused by poverty resulting from unemployment or underemployment. India, for example, over the last 15 years, has produced more than an adequate supply of cereal grains. As a matter of fact, it has in recent years maintained in storage something on the order of 20 to 25 million tons of wheat and rice. But there is still hunger and malnutrition in India. Indeed there are many people who need more food, but cannot afford it even though it is present in large quantities within the country. I do not wish to imply that there should not be stocks of food stored as a hedge against bad harvest, epidemics, or natural disasters. Indeed, we must continue to maintain buffer stocks that can be used in times of crisis.

Let us examine what has happened in the last 15 or 16 years. In 1972 and '73, the large U.S. grain stocks that existed during the late 1950s and throughout the 1960s, resulting in depressed prices for American and other farmers, suddenly were depleted. This sudden, rapid and unexpected change was caused by severe droughts in two consecutive years in the Soviet Union and China, which resulted in large imports of grain by those nations. With reserve stocks depleted precipitously, rapid price increases ensued. Outcries against soaring food prices came from urban consumers in the United States, who were used to cheap food. The general public, and especially many farmers, listened with too much credulity to the predictions of gloom and doom that were presented at that time by several distinguished academicians armed with the best computers. They predicted that the world had lost its ability to feed a growing population numbered at four billion people at that time.

What were the consequences of those predictions? It is my belief that many farmers and economic policymakers involved in agricultural decision making overreacted. They immediately put back into production virtually all of the land that had been held out of production for a decade because of surpluses. The U.S. Secretary of Agriculture recommended that farmers plant from fence row to fence row, implying that the international market would require all that could be produced. The situation was made worse about three years later when a huge jump in the international price of petroleum caused a vast inpouring of petrodollars. Those petrodollars were siphoned down to rural banks and resulted in easy credit. Many fine young farmers, tempted by that easy credit, decided that if the world had run out of its capacity to produce the food that was needed for our four billion people, as was predicted, it was time for them to buy more land. This was easily done by

signing mortgages on the property they already held. The demand for land and easy availability of money led to speculation and soaring prices. Midwest farm land prices increased from $800 an acre in the early 1970s to $3,500 during the early 1980s. Vast investments were made to stimulate food production which increased spectacularly, resulting by 1983 in huge grain gluts. Then prices collapsed. Many farmers who overzealously expanded their family farms were deeply in debt. Many of them lost their farms when the agricultural commodity price bubble broke.

At the same time that surpluses were building in the United States and other exporting nations, there was widespread famine in Africa, in part because of the extensive drought. But beyond the immediate cause, for the last two and one-half decades, agricultural production had been increasing in the African countries south of the Sahara at about one and one-half percent per year, while population had been growing at about twice that rate. During the drought years of the middle 1980s, food demand outpaced production, resulting in widespread food shortages. Even after the drought is broken, it is doubtful that, with the agricultural technology now being used, African countries south of the Sahara will be able to catch up with the food demands of the rapidly growing population.

From 1973 to 1986, a similar situation on the energy front also affected agriculture. In 1973 and '74, the petroleum exporting countries (OPEC) arrived at an agreement whereby they could control production. The price of petroleum then soared from about two dollars a barrel to 11.65 then to 18, then to 28, and finally to 32 dollars a barrel, within a period of three years. This huge increase in petroleum prices also generated songs of gloom and doom by some experts and academicians who claimed that the world had lost its ability to exploit petroleum and other energy resources. A short time later, predictions were made that the world was running out of many basic mineral resources as well. Again, in terms of energy and minerals, the world overreacted. From '74 to '86, huge investments were made in petroleum exploration and developing new fields, in re-exploiting depleted fields, in substituting other energy sources, such as coal, gas, and atomic energy, and in conserving energy through more efficient transport systems. By late 1986, all of these endeavors led to a huge buildup in petroleum production and a collapse of prices.

During the petroleum shortage and skyrocketing prices, the huge influx of petrodollars into American and Western European banks led to a dilemma for many bankers—where to invest these funds? International bankers went to Third World nations, especially countries that had underdeveloped petroleum deposits, and encouraged

them to take out huge loans. The developing countries were led to believe that then current petroleum prices of 28 to 32 dollars a barrel would soon increase to 60 dollars, since the supply of petroleum was dwindling. Huge loans at high interest rates were taken out by many Latin American and African countries. This resulted in economic crisis in December of 1986 when the world glut of petroleum caused prices to plummet from 32 dollars a barrel to 11 dollars in a few months.

During the period of high international petroleum prices, many other developing countries had to import petroleum even though they were very short of foreign exchange. They were forced to take out large loans to buy energy and, as a result, are currently in dire economic straits. They are short of foreign exchange; they are deeply indebted; they have rampant inflation; and many are short of food. In real value, the money available for agricultural research today is one-half, and in some cases only one-third, of what it was eight to ten years ago. Consequently, most Third World nations have retrogressed in their ability to improve and expand research to cope with worsening food shortages. How should these countries allocate the available funds? How much should go to conventional agricultural research across the various disciplines and how much should be allocated to research in biotechnology and molecular genetics? Clearly, applied research in scientific disciplines that directly bear on food production must be given major short-term emphasis. Unless much of the widespread poverty, hunger, human misery and inflation is ameliorated in the next two decades, greater and greater social and political instability will occur in the world, particularly in the developing nations. The attack on these ills must be aggressively launched with the conventional technology now available. This offensive need not wait for developments in biotechnology.

What needs to be done to improve production and distribution of food in Third World food-deficit nations in the next decade? For the last two decades, with the assistance of international centers, bilateral foreign assistance programs, and their own national programs, many of these nations have developed important bits and pieces of the jigsaw puzzle of food production in different disciplines that bear on improving agricultural productivity. This has been done by conducting appropriate research in most of the disciplines that are keys to changing yield and production. What has not been done, in most cases, and must be done if there is to be a change in production, is to assemble these bits and pieces of the production jigsaw puzzle into an appropriate package of production technology and evaluate its benefits and risks on many farms. To achieve the potential research benefits, a few courageous,

creative individuals across scientific disciplines are needed who can integrate the puzzle pieces of food production technology and visualize its potential to reduce hunger by augmenting food supply when the package of technology is properly assembled, adequately evaluated, widely demonstrated on farms, and finally broadly adopted by farmers. Unfortunately, research programs in most developing nations (and also in developed nations, where this luxury is more affordable) have "chaos in the brickyard"—too many bricks in some disciplines and few or none in others. Hence, the research package is incomplete and it is difficult to assemble a reliable production technology package because of the risks engendered by research gaps.

Once the production package of technological bits and pieces is assembled, it must be tested first on dozens of farms and then over the next two years on hundreds and even thousands of farms, with additional adjustments as improved experience and data become available. When the package is properly assembled and widely demonstrated on many farms, the enthusiasm of both big and small farmers will be fantastic—literally "will set the grass roots on fire." Farmer enthusiasm is infectious and affects politicians, policymakers, and even some of the immutable bureaucrats. After only two years of recent work in three African countries, I sense an enthusiasm, especially in Ghana, that is fully the equivalent of what we saw in the middle 1960s in India and Pakistan, when the so-called Green Revolution, based on the high-yielding wheat and rice technology, was transplanted into those countries.

Once the capability of the new technology package to increase yields spectacularly has been broadly demonstrated, credibility has also been established by gaining the enthusiastic support of thousands of farmers who know what is technologically possible. It is then time to move on the economic policy front and it is usually possible to convince policymakers and political leaders that unless policy errors of the past are rectified, social and political problems will increase in the years ahead. Economic policymakers must be convinced that the right kind of production inputs must be made available in a timely manner at the village level, that small farmers need access to credit with which to buy the inputs for planting and can pay for them at harvest, and that the government must announce before planting that the farmer will receive a fair price for his grain at harvest, which fairly reflects the international market price. Moreover, to avoid local gluts that will depress the market price, the government must provide for procurement in surplus production areas at the time of harvest.

Increased production that theoretically meets local needs is not enough. Action must also be taken to get the food into the stomachs of the poor, those who need more food and are badly nourished. This requires examination and use of many devices. There must be rural development projects to develop the infrastructure and provide work for many of the 75 to 85 percent of the population who are living at or near subsistence level in rural areas. There should be food-for-work projects to develop roads, build country schools, and all sorts of soil conservation, small-scale irrigation and drainage projects, and reforestation where possible.

Is there technology available that lies unused, which has the potential to change African food production over the next five to eight years? For the last two and one-half years, I have been involved in developing small agricultural demonstration-production programs in three African countries, financed by Ryoichi Sasakawa, a Japanese philanthropist. This program, known as Sasakawa Global-2000, was launched after much pressuring. I had twice retired and decided that I had served my term on Third World food production fronts, and that I was never again going to become embroiled on the front line in these kinds of programs. Under pressure, I agreed to do so once more, against my better judgement. Surprisingly, after only two years, I am now convinced that there is an excellent chance for a breakthrough in both maize and sorghum production in Ghana within the next two years, within the next three or four years in Zambia, and also, perhaps in Sudan.

In the broader global picture, I am convinced that the world now has the technology available to produce adequate food which would, if properly distributed, provide an adequate diet for our present population of 5.1 billion people. Perhaps the technology now available can even produce the food required for 6 million. Nevertheless, without a more effective system of equitable food distribution to supplement the improved production technology, social, economic, and political problems will worsen.

I am pleased to see that vigorous research is going forward in the new field of biotechnology which, down the road, whether it is in 10 years or 30 years, can be used as needed to greatly expand the production potential of food and fiber.

The most productive applications of biotechnology and molecular genetics, in the near term, appear to be in medicine, animal sciences, and microbiology. Improved vaccines, antibiotics, and enzymes and hormones for the treatment of genetic defects are already beginning to appear on the market. It will likely take considerably longer to develop biotechnological research techniques

that will dramatically improve the production of our major crop species. We should view biotechnology as another new tool that will further help to protect mankind from diseases and pests and help increase food and fiber production, rather than to believe it will be a panacea.

Many environmentalists or neoecologists fear the use of modern technology. We should all stand back and look at what the world situation would be like today if we attempted to produce the food and fiber needed for the current 5.1 billion world population with 1930's technology which was, for the most part, prechemical. The technology of 1930 provided food and fiber for a world population of two billion. It would be completely inadequate to provide the present standard of living for the much larger present population and the need to correct the deficits of those who needed more food and fiber and other benefits for a decent life. Let us continue to work diligently to improve the research and technology in our chosen specialties—"looking enthusiastically through our individual knotholes"—while endeavoring to untangle more of nature's secrets, which we will ultimately need to understand, to further improve the standard of living of more of the human family.

We must all be realistic about what life is like in the Third World nations and what actions are necessary to provoke change and improvement. Having the technology is not enough; it must be put to work. Someone has to be responsible for making it work. And that is no easy task.

Biosafety In Agricultural Research

Bruce L. Umminger
National Science Foundation
Washington, DC

Biosafety Guidelines in the Developing World. Dr. Henry Miller, facilitator for the workshop, opened with the observation that biosafety is an important attendant to science and technology. Governments give and governments take away. Funding agencies do the former and regulatory agencies do the latter. In the developing world, the regulatory infrastructure is less developed, in part because such regulation is expensive to develop and to enforce. To give more specific insight into the state of biotechnology regulation in the developing world, three speakers summarized the current state of biosafety regulations in their countries:

The Philippines. Dr. Delores A. Ramirez summarized the recent efforts of a committee of scientists from the University of the Philippines, the International Rice Research Institute, and of administrators from the government to develop a set of biosafety guidelines intended to set safety standards at their respective institutions. The *ad hoc* committee did not start from scratch, but adapted provisions from the U.S.A.'s NIH guidelines, Australian guidelines, and Japanese guidelines to the research conditions of the Philippines. The Australian and Japanese guidelines were especially helpful in areas of evaluation whereas the NIH guidelines were most useful in the area of containment. Although the guidelines used as models dealt mostly with recombinant DNA, the Philippines guidelines were conceived as being much more broad and include procedures to complement and supplement existing plant quarantine laws. After completing the draft guidelines, intended originally for local use only at the University of the Philippines and the International Rice Research Institute, the committee deliberated as to whether these could be used at the national level. To ascertain the wider usefulness of the draft guidelines, a workshop will be held later in 1988. The workshop will be sponsored by the National Academy of Science and Technology, the University of the Philippines, the Department of Science and Technology and the Department of Agriculture, among others. At the same time, Philippine legislators became interested in the question of regulating biosafety. The legislators have been asked to avoid new legislation until the results of the workshop are known and then, if legislation is deemed necessary, to use the draft guidelines as a starting point for biosafety laws.

The draft guidelines consist of three major parts: (1) evaluation of research with recombinant DNA; (2) evaluation of research involving the importation or breeding of plant pests and research with potentially hazardous organisms (to supplement quarantine laws); and (3) procedures for containment for recombinant DNA research. The guidelines were drafted to reflect the state of affairs in the Philippines and are viewed as imperfect, but evolving guidelines that will change as experience warrants.

There is also to be established a national committee to oversee all aspects of biosafety—the National Committee on Safety in Biological Research in the Philippines (NCSBR). The NCSBR will operate under the Department of Science and Technology and will have linkages with the Departments of Agriculture, Environment and Natural Resources, and Health, among others. Institutional Biosafety Committees (IBCs) will be established at each university and research institute. The system will operate as follows: a university researcher will have his project reviewed by the local IBC which will then make recommendations to the NCSBR. If the project involves importation of plant pests, for example, the NCSBR will forward project to the Philippine Quarantine Service for final permission to do the research. Linkages to the other departments will also be used as appropriate.

The NCSBR will have seven members and will be chaired by the Undersecretary of Research and Development of the Department of Science and Technology. Four members of the committee will have at least seven years of college/university research experience. No conflicts of interest with industry will be allowed.

Functions of the NCSBR include the formulation, review, and oversight of biosafety guidelines; the formulation, review and oversight of risk assessments for biotechnology products; the development of working linkages with the Department of Agriculture, Environment and Natural Resources, and Health; the development of technical capability in the country; the development of research programs to establish risk assessment protocols and assess long-term environmental and social costs of biotechnology; and the appointment of IBC members. The proceedings of the committee will be made public.

Functions of the IBCs include the review of all projects at the local level and recommendations to the NCSBR; the assessment of appropriate containment levels for projects; the review of all the biological researchers in the institution; the review of plans for handling accidental spills, etc.; the reporting of violations of guidelines to NCSBR; and the rejection of proposals not meeting

guidelines. The IBCs will have five members, two of which are not affiliated with the institution and represent community interests.

The guidelines are proposed, but not yet adopted. Supporters of the guidelines include research institutes and universities. Those in favor of more restricted guidelines include environmentalists. The focus of the NCSBR will not be on recombinant DNA *per se*, but on potential pest organisms. In addition to R-DNA organisms, all plant pathogens, introduced biological control agents, and "potentially hazardous organisms" will be reviewed by the NCSBR. The definition of "hazardous" is fuzzy at the current time. It is a product-oriented and not process-oriented regulatory apparatus. Nonetheless, all recombinant-DNA genetically engineered plants will have to go through the NCSBR without exception; in this sense, the system is process-oriented.

Thailand. Dr. Sakarindr Bhumiratana summarized the situation in Thailand. The regulatory system in Thailand is much more local, decentralized, and informal than the one proposed for the Philippines. In Thailand there are two aspects to biosafety: regulation and enforcement. Thailand already has numerous biosafety regulations in place with respect to biomaterials, human experimentation, quarantine regulations, etc. However, the regulations only can be as effective as their enforcement. Currently, there are no regulations for recombinant DNA *per se*, even though several labs are engaged in this type of research. There is some fear that if biotechnology regulations are formalized, the recent science and technology thrust of the country will be stalled in this area. At present, each university and institute has in place its own local committee that operates in a rather *ad hoc* man[illegible] according to the NIH guidelines. The local committees hav[illegible] information on guidelines from the United States, Austra[illegible] Japan. There are no formal national regulations or committees for biosafety. Thailand is not overly concerned about setting up new laws and regulations since the country has only a couple dozen researchers in this area and their research should not be thwarted. Nonetheless, a couple of study committees are being set up to look at field trials due to some public concern. Those guidelines that are used at the local level are mostly process-based, i.e., concerned only with R-DNA research.

With this system of self-regulation at the local level and case-by-case evaluation, there have been no problems so far. The National Center for Biological Control, for example, has many practicing scientists and no experiments have been performed that have caused a problem. Thailand has approached biotechnology as a nation from a positive side. The benefits of the research have been made known to the public at large. Government ministers have

made a good case for science and technology in general and biotechnology in particular. The public has been sold on its value; environmentalists have not yet voiced concerns.

Africa. Dr. Wellington Otieno of Kenya gave a generalized overview of the state of biotechnology regulation in Africa. In general, there are three levels of concern with biosafety: (1) at the quarantine level for the importation of seeds, plants, etc. between nations and continents; (2) at the biological control level for parasites, microbes, pathogens, etc., imported to control pests; and (3) at the level of genetic engineering or R-DNA. The former two levels have quite strict regulations whereas the latter oversight is at the local level by informal oversight committees at the universities. Not many universities have expertise in molecular biology and R-DNA, and so extensive regulatory statutes have not yet been required. The public is not overly concerned. For the quarantine and biological control regulations, there is a broad degree of harmonization of regulations among African nations. Although there are not now any specific R-DNA regulations at the national level, broader guidelines will most likely be developed in the future in the context of interacting African nations. Likely the guidelines, when drafted, will be based on local institutional guidelines and those existing guidelines in the international community. At the present time, there is the danger that if overly restrictive regulations were introduced, they would choke off the developing biotechnology research enterprise that does exist. The general approach of African nations is pragmatic: if the product being produced is good for the people, then don't cut it off with regulations.

Discussion. The general discussion that followed the three presentations fell into three main areas: guidelines, public education, and information/data bases.

Guidelines. It was pointed out that guidelines developed in the Third World should be flexible since, even in the developed world, regulations and laws are in a state of flux and development. Don't put into place regulations that cannot be easily changed in the future as conditions and experience warrant. It is the place of donor agencies to help developing nations get regulations in place that will allow solid research and development and protect against mistakes in judgment. Many stressed that developing countries cannot wait until the developed world has a final set of regulations; there are immediate problems now. The best course is to develop regulations based upon those already existing in the developed world. As in the Philippines, the guidelines can be tailored to the specific national needs and circumstances. If the guidelines are flexible, they can be changed in the future. Indeed, it was pointed out

that developing countries should not reinvent the wheel. Especially useful are the beginnings of international biosafety regulations being worked out by such groups as UNIDO/UNEP/WHO in the UN system, SCOPE/COGENE in the ICSU system, the IICA for Latin America, NATO, and the OECD, among others. All these efforts are consistent with the philosophy and point of view of a report by the U.S.A. National Academy of Sciences that stresses a product-based approach to biosafety regulations and not a process-based approach. ICSU was said to be holding a conference later in 1988 to help developing countries set up their own guidelines for biosafety. It was stressed that too many international groups were setting up guidelines that were duplicative; there should be an international effort to coordinate this undertaking. The forthcoming ICSU conference should be coordinated with other international efforts. A goal of donor agencies should be to see that such international efforts in drafting biosafety guidelines, especially as they apply to developing countries, be coordinated, consistent, and science-based.

Public education. There was unanimous sentiment that a goal of donor agencies should be to see that the public is well informed in the area of biotechnology. This should include education in the areas of ecology and risk assessment. Important targets for this education are politicians and the media as well as the general public. A climate should be created such that when commercial biotechnology comes on line with products, the public is well informed and not unduly worried. The education effort should be honest and acknowledge those risks that do exist, but point out that these risks from biotechnology are very similar to the risks that we encounter in organisms we already know a lot about. There may be some risks, but they are not unique. In developing countries, it is often the extreme environmentalists and those opposed to any foreign-introduced technology that are in most vocal opposition to biotechnology. Thus, the need for public education should include ecology and risk assessment. In the area of risk assessment, the public should learn to think in terms of the real benefits produced by the technology and to evaluate whether these offset known risks. Collaborative approaches with donor agencies could get this accomplished. A value-neutral approach is essential.

Information/data bases. It was generally agreed that developed countries and donor agencies should make more effort in getting information disseminated to the Third World. This often entails more than just access to electronic and computerized data bases that are being planned. It was generally agreed that developing countries are somewhat suspicious of information supplied directly by a given developed country such as the U.S.A. The best ways to pro-

vide information are through the UN or a local science development board or center. There is a great need for an information clearinghouse on biosafety information for the developing world. The consistency of the recent UNIDO/UNEP/WHO guidelines with those developed by other groups in the developing (IICA, ICSU) and developed (NATO, OECD, the National Academy of Sciences) world make the UN a possible place for such a clearinghouse to be located.

It was also suggested that information exchange outside the context of national governments could be effected through the academies of sciences in various nations. The impact and importance of the recently organized African Academy of Sciences was noted as an example of multinational cooperation in the developing world. The International Centers are also good avenues for exchange of information as they are generally viewed as neutral bodies.

Application of Diagnostics for Plant and Animal Pathogens

Joyce Turk, J.I. Cohen and D. Luchsinger
Office of Agriculture
Agency for International Development
Washington, DC

The diagnostics workshop combined opening statements by representatives of various biotechnology subsectors with full discussion by all participants. Opening statements highlighted the following needs and opportunities: (1) identify high priority diagnostic requirements, (2) communicate how these technologies can be applied, (3) recognize that tools currently available for legume viral diseases are not adequate, (4) no immuno-diagnostic tools are available commercially for fungi and bacteria, (5) commercial firms are interested in biotechnology collaboration opportunities, and, (6) donor agencies could serve as a vehicle to transfer much of this information.

Open session dialogue encompassed research and development, commercialization and marketing of plant and animal diagnostic and vaccine products. Discussion from the floor began with comments from Kurt Peters of ILCA. He believes the greatest need of the livestock sector is getting diagnostic tools, including those for endoparasistes into the hands of users. A question was raised concerning the advisability and acceptability of the collaborative venture in diagnostic development at ILCA. Is there a comparative advantage for such a venture and how could commercialization and marketing be developed? Opening remarks captured the range of complex issues being faced, i.e. awareness of the need for the development of diagnostic tools by researchers or research centers, linkages with the commercial firms for development, advertisement, marketing either in the country of origin or a second country. A great deal of discussion related to one aspect or another of this loop from awareness to application.

It was recognized that international organizations could play a vital role by developing catalogs of recognized needs and opportunities in diagnostics. The commercial sector response could be to publish and distribute information regarding current production. A mechanism to link available research products in the public sector with recognized international needs could also be established. If markets are identified, then industry can respond, if not, researchers and donor agencies can respond, were points made by T.D. Yilma.

Considerable discussion followed regarding linkages. To begin, information exchange between the public and private sector needs to be increased. This will help to match what is needed with what is available. A mechanism is requried which can develop personal bonds of collaboration encompassing interests, trust and expertise. Individual lists of needs regarding diagnostic research, based upon the size of the problem and how widely applicable a diagnostic tool would be, should be developed and presented to donor agencies.

A collaborative venture between international centers, universities and commercial research could develop multiple screening tests and capture a larger market than originally envisioned. Marketing was taken to mean primarily dissemination of information and/or royalties. Such interests must be combined with those of making technologies available to areas where diseases are severe problems. However, it was recognized that universities do not necessarily have an interest in marketing products of their research. A suggested recommendation was that A.I.D. put out contracts for commercialization based upon research it is funding.

A question of interest arose regarding the development of DNA probes in relation to subunit vaccines. Is there an ability to commercialize these and what is the interest in supporting commercialization?

A company such as AgriTech Systems looks for such commercial opportunities. Users must understand the costs involved in commercializing diagnostics. In some cases, it may be better to manufacture the kits in the user country. This company currently has production in 35 countries and is especially interested in licensing technology for codevelopment. There is a definite interest in working on diseases not present in the U.S. according to Dr. Workman.

Another recognized objective was to develop cost-effective delivery mechanisms for varied market sizes. The use of diagnostics will not be cost effective if they have to be imported. Local companies must be developed which can either produce or buy reagents in bulk and can do local packaging. Currency restrictions may inhibit the ability of a country to import kits.

A different type of need relating to plant quarantine issues was recognized, that being, the ability to detect plants transformed by genetic engineering. The discussion shifted to diagnostic concerns regarding plants and included the following observations. Nonradioactive probes are needed where no other assays are available. There was no clear consensus as to whether probes

would be advisable over immunoassays. There is a need to produce simple, cost-effective methods which would replace the ELIZA system. International donor agencies and IARCs should support such field research. In this regard, another challenge is to develop kits which provide reliable information from a small number of samples. Diagnostic tools for crops do fit into traditional farming systems. However, farmers will need to know when and how diagnostic tools can best be used.

The workshop closed with many unanswered questions but with those in attendance more enlightened and aware of the vast number of opportunities for future research and collaboration.

Workshop On Wide Crosses and Genetic Resources

Robert B. Bertram
Food and Agriculture, CGIAR
Agency for International Development
Washington, DC

Plant and Gene Patenting

The Workshop session featured in-depth discussion of several themes that had been brought out in the plenary sessions. The issue of gene patenting, which surfaced in many of the discussions, was examined in detail by representatives from both developed and developing countries. The fundamental challenge was how to reconcile traditional concepts of free exchange and availability with the protection sought by institutions seeking a just return to significant investment to develop new and novel products. There was a general consensus that naturally occurring genes are a "heritage of mankind," and as such should not be subject to patent protection. Any patenting of naturally occurring genes would threaten the free exchange of plant germplasm, a basic tenet of the current international germplasm system and a critical factor underpinning agricultural research everywhere.

Conversely, the issue of payment for naturally occurring genes was raised. There was general agreement that such proposals were unworkable. First, determination of actual provenance of a gene in most cases would be extremely difficult, so that it would be very difficult to assign ownership rights on a scientific basis. A second concern is that many important genes had been moved and modified frequently over centuries of trade and migration. Examples were given of genes which might be collected in one country, but were actually selected for in a country far away, from which farmers migrated hundreds or even thousands of years ago. These realities, compounded with shifting boundaries and sovereignties render the idea of payment for naturally occurring genes as something quite impossible to implement. Further, would farmers themselves be paid (presumably for the efforts of their ancestors), or would governments act as recipients? However, the point was made that the impetus for assuring genetic rights of developing countries to land races, and products bred from them, stems from a desire to protect their right to use such material, which they fear could be sold back to them by foreign interests.

Besides the historic and political issues that would arise over assigning payment rights to naturally occurring genes, there is also

a significant knowledge gap in what would be needed to actually identify and track them. Variable phenotypic expression of genes is noted in multiple environments and presents a major challenge to plant breeders. The resulting variation makes consistent identification of a single gene very difficult. The task of assigning payment rights would be further complicated by the fact that many genes are found in several areas, each of which might claim to be the area of origin with proprietary rights. It was also noted that, contrary to what is widely believed, much of the valuable genetic diversity of crops has come from regions in developed, not less developed, countries. Thus, schemes for payment based on genetic rights, if applied without regard to need or ability to pay, could affect both developing and developed countries.

The question of gene patenting was acknowledged to be relatively new to the conservation and agricultural research communities, although plant variety protection has been established for many years. Still, issues surrounding patenting are different enough that they need to be approached independent of prior assumptions. Some participants pointed out that patents can be viewed as generally benefiting the "first world," and are considered to hinder plant breeding progress in developing countries. If this is the case, greater opposition to patents would occur. However, the argument is also made that appropriate patent protection will speed the distribution, use, and hence, accessibility, of proprietary germplasm to developing countries.

Some guidance was offered by the legal expert, who noted that from the perspective of the law, a gene is a chemical compound. If it occurs naturally, it probably cannot be patented, because no one invented it. It would fail the basic test of novelty, upon which patent law is based. But even in cases where novelty were proven, there would still be difficult questions that have yet to be answered. In some cases, the U.S Patent Office will grant a patent on a naturally occurring gene, provided that the gene is isolated or modified. In doing so, the Patent Office is building on cases in which microbes have been patented after being isolated from culture and produced in the form of pure strains.

The question of genetic instability and "genetic wobble" was also raised, as this results in changes in genes over time. How would patents account for such changes? Similarly, the question of how far a patent would extend was posed. For example, if a farmer purchased seed containing a patented gene, and then multiplied it for his or her own purposes, would that constitute patent infringement? Seed companies must envision reuse and price accordingly. The

critical issue is whether a company can keep a farmer from reselling the seed as seed. With regard to enforcement, it may become possible to use cDNA or mRNA probes to detect potential infringement.

Another controversial aspect of plant and gene patenting that cannot be ignored is the impact it is having on overall dialogue between developed and developing countries. For many, this is not a financial but a moral issue, upon which hinges progress in developing countries' alleviation of hunger and maintenance of competitive agricultural sectors. The introduction of patent legislation in general has the potential to generate hostility in certain sections of the international community, and steps should be taken to avoid this.

The International Agricultural Research Centers clearly will have a role, both as users and providers of germplasm, in any legal arrangements that emerge. The question was raised as to whether the IARCs should seek to guarantee free availability through patenting of novel genes, and then ensure the access of developing country researchers to them free of charge. An additional feature of this idea would be to obtain royalties for use of such materials by commercial firms or by developed countries. Others suggested that even this approach to patenting would engender controversy and harm the Centers' collaboration with national programs.

As an alternative to patenting, the example of copyrighting was suggested. Copyright law protects information (generally written), which is in fact what is carried in a gene. The protection of newly created genetic information in this manner might avoid the difficulties minor genetic changes (for example, a nucleotide base substitution) would pose to patented materials. How copyright would govern further use of materials in commercial breeding was not clear. It has not been used extensively in genetics, except by one firm developing a map of the human genome.

The point was made that it is not simply single genes that are at issue. A gene does not operate in isolation, and some of the most challenging scientific problems facing researchers hinge on gene regulation through provision of appropriate promoter and enhancer sequences. Processing of gene products may also be crucial to their biological activity and hence, commercial worth. Overcoming such challenges associated with creating new genes or genetic transformation obviously requires significant investment. Clearly, the private sector, and increasingly, developed country public sectors, will undertake such investment only with some assurance of financial return.

The point was made that a gene's function and worth are also dependent upon the background in which it is found. Even new, engineered genes are of little intrinsic worth without an appropriate genetic background, which most likely is entirely made up of natural genes, painstakingly sorted through hundreds of generations of farmer selection or through the direct efforts of plant breeders. From this standpoint, assurance of free exchange of genetic materials is seen not only as being of necessity to plant breeding progress, but also as a morally derived right.

Interestingly, the principal examples of germplasm having been restricted are among tropical crops, which in some cases are limited by developing countries. Most of the affected crops are those of large economic importance, e.g., coffee, cocoa, etc., and not the food crops that have been the focus of the International Centers' efforts.

The discussion did not resolve issues surrounding patents, but it did make abundantly clear the need for sound, up-to-date information in the development of laws and policies related to genetic resources. Availability of genetic resources is a goal shared by all plant scientists. Reconciling this basic tenet of the international system with development of patent laws, now in effect in developed countries, but most likely to be activated soon in developing countries as well, is an important objective. Scientists should be involved in the development of the patent process, ensuring that it proceeds with as full a body of information as possible. Patent regulations should, as far as possible, be consistent with biology.

There was clear agreement that free access to naturally occurring genes should not be limited, but a basic question here was: how can we ensure this? Up until now, individual and institutional commitment, combined with good will on the part of far sighted scientists, have been sufficient to allow for a well-functioning system. The question of whether new approaches to ensuring free exchange of naturally occurring genes will become necessary as the body of law governing biotechnological research products enlarges was left open. In the view of some present, the situation calls for vigilance on the part of the genetic resources community.

Wide Crosses

The Workshop group explored the issues raised by plenary session presentations on the use of wide crosses in groundnut and wheat improvement. The group generally saw rapidly developing potential from the application of biotechnology to wide crosses and crop

relatives generally. Biotechnology may help overcome crossing barriers, and it may provide markers with which to track and select desired genes once crosses are made. As nontraditional methods to transfer genetic materials develop, interest in wild species will increase, in some cases with impact beyond their near relatives. Tempering the discussion was the observation that wild species work should not become a "bandwagon," risking their being oversold as a source of valuable genes. Nevertheless, there was a call for a greater allocation of international resources to the area, since in many crops very little had been done with wild relatives.

A theme that ran through the discussion was that strategic use of wild relatives of crops was needed. The observation was made that exhaustive searching of the domestic species should be undertaken before seeking a particular gene from a wild relative. In many cases, land races or other primitive materials may hold untapped diversity much more easily accessed than that in materials outside the species.

A recommendation was made that IARCs establish research networks to foster trait-specific work in wild relatives of mandate crops. Networks were seen as very important because of the need for collaboration in working with wild relatives—those knowing such species need to work with those collecting and storing them (germplasm units), and those seeking to use them in breeding programs may need close collaboration with biotechnology laboratories. Centers could help to identify and bring together scientists with complementary interests through such networks; opportunities for interdisciplinary collaboration are not always evident and need to be developed. There was also a suggestion that the interests of the research community must expand to include biological diversity in general.

With a broader definition of biodiversity in mind, the potential for collaboration between developing and developed countries appears very substantial. Three major areas for joint work are seen: food crops, ornamental plants and medicinal plants. In the cases of medicinal and ornamental plants, the possibility of profits will help to ensure that efforts are undertaken; collaboration should be an important part of these efforts. In the case of food crops, there may be less financial backing to support collaborative research involving wild species. What is needed is for collaboration to begin from the bottom up, with researcher to researcher contact and interest compensating for the lack of major profit-driven investment.

Certain caveats were given regarding work with wild species. First, very large populations are needed to effectively sample variability

for characters of interest. Even where a character is identified, the logistics associated with handling the species and accessing the character genetically can be extremely difficult. To access genes for resistance to just a single pathogen, a concerted and painstaking effort is often required. This argues that strategy when dealing with wild species is extremely important. Chromosome transfer is seen as a promising alternative to genomic transfer. Much less material is moved, greatly facilitating the reestablishment of good agronomic standards among the progeny. The use of chromosomes or fragments offers good possibilities for collaboration among researchers, bringing cytogeneticists, breeders, botanists and plant biotechnologists together.

The point was made that, especially in the case of wild species, there is still considerable ignorance regarding their taxonomy, distribution and characterization. Collaboration between disciplines is required here. For example, in the experience of the IBPGR, breeders are not generally thought to be the best collectors of wild germplasm. The IARCs that have been active in this area have developed extensive collaboration in order to most efficiently pursue wide cross research programs. In terms of utilization, the potential for national programs to be active in the wild species/wide cross area seems very great. National program/IARC collaboration would certainly figure prominently, and the question was raised as to whether there could also be linkages to private research. Specialization in enhancement, introgression and general characterization could become an area of special expertise for national programs. National programs would also be well situated to conduct prebreeding activities built on wide cross programs. Clearly, though, the field is moving quickly, as there has already been patenting of the process used to introduce a chromosome from *Tripsacum* into maize lines.

In the case of crop gene pools, evaluation of germplasm remains the major bottleneck facing wider use of genetic diversity. The Latin American Maize Program (LAMP) described by Quentin Jones offers an excellent opportunity for future collaboration. After two years of evaluation and selection, breeders will begin to exchange materials that have been selected for particular traits. The project features a large informational component, so that all researchers are informed of what others are observing in the materials under their study. All countries participate in designing the activity, and all have access to the materials it generates. This method of achieving broad-scale evaluation and sharing of information and germplasm could serve as a model for other crops.

Collaboration for mutual benefit has been the driving force behind the maize RFLP (restriction fragment length polymorphisms) network that is developing in Europe and the Americas. The basis of activity is that each institution will study a single trait, the results of which will be shared with all other participants in the network. CIMMYT will act to ensure that LDC programs have access to, and benefit from, all the information generated by the collaborators.

Researchers emphasized that sharing of information based on mutual benefit can go a long way towards addressing concerns over access. This collaboration, much like the germplasm system, must be based on mutual trust.

Molecular Technologies

David L. Edwards
Western Boitechnology
Del Mar, California

The form presentations which comprised the Molecular Technologies formal session provided the following important information:

- Genetic engineering of plants for insect resistance, virus resistance and herbicide resistance has been demonstrated through the field trial stage. The technologies are now well established and will have broad application.

- RFLP analysis is still in the developmental stage. It may be very useful for well-studied crops such as maize but at this point the experimental technology is cumbersome and may be difficult to establish in a developing country on a relatively unstudied crop.

Workshop discussion centered on the following points·

Intellectual Property Rights

Private companies that have developed proprietary crop strains need to recover their research and development investment through the increased price of the value-added product. This becomes a point of concern in cash-poor developing countries. Many of these countries also do not provide protection for the patents held on such products.

The companies present all expressed a willingness to discuss various means of recouping their investment from developing countries which would benefit greatly from the technology. Some of the possibilities discussed were direct licensing, bartering for natural resources or other such *quid pro quo* arrangements. The question was raised as to what value to put on a gene (or a trait) and how much compensation the owner might be entitled to.

The role of the International Centers in facilitating these exchanges was discussed and it was proposed that an international conference be set up to discuss how to proceed in these matters.

Collaboration on Virus Resistance Projects

It was proposed that a networking arrangement be developed to produce virus-resistant varieties of plants critical to developing

countries: cassava, common bean, peanuts, etc. This arrangement would include:

- Characterization and molecular genetics of the causative agent (cloning, capsid protein genes, insertion into plant of interest).
- Testing and propagation of resistant plants.
- Distribution to users.

Professor Beachey and his group at Washington University, St. Louis, MO have volunteered their facilities for the initial molecular characterization and cloning studies.

Mutuality

A long discussion evolved around the fact that developed countries have technology while many developing countries have an abundance of natural resources. Developing countries must find a way to gain value from these resources which can be used to access needed technology. For example, valuable genes (*Bacillus thuringiensis* toxins, resistance genes, etc.) might be discovered in developing countries and traded to developed countries for established technology. It was also suggested that developing countries develop some of their own commercial enterprises (Third World seed companies) to exploit their own resources. These entities could then become vehicles for licensing technology from their developed country counterparts.

The legal issues of ownership of genes and rights to technology must be clarified in these cases. For example, technology might be transferred to a developing country for a small royalty based on the country's ability to pay and an agreement that the developing country will not resell the technology back to the developed world. The International Centers might act as facilitators in such transactions.

Research Needs of the Developing Countries

Among the research needs mentioned as priority items for the developing countries were:

- Toxin genes active against Third World insects: grasshoppers, locusts, aphids, and nematodes.

Agency Support for Research Projects

It was pointed out that many crops utilized in the developing countries have no real value in the developed world and, therefore,

there is no compelling reason for developed countries to put resources into improving these crops. Cassava was given as an example. It was suggested that an important role for the International Donor Agencies would be to fund research and development activities on such crops.

Plant Cell and Tissue Culture

Oluf Gamborg
Tissue Culture for Crops Project
Colorado State University
Ft. Collins, Colorado

The workshop attendees discussed opportunities and ideas for initiating and establishing collaboration between private research institutions and international research centers. They agreed that contacts could be initiated through individuals in a manner that will establish mutual trust. Areas of common interest, particularly those in which proprietary issues are minimal, should be explored with training as a possible starting point.

The comparative advantages and limitations of private sector organizations were the next topic of discussion. Participants noted that while private institutions are flexible and willing to discuss a wide range of issues, they are more likely to become further involved only when there is a prospective payoff. Private sector entities can provide information and expertise that is of value to international centers. They can offer training, and advice and assistance in the development of products and technologies for larger-scale and commercial use. A greater awareness of the scientific potential in developing countries can be developed through regular private sector contacts with international and national centers. Private organizations should also be the source of scholarship and stipends for deserving students and trainees from developing countries for in-country and overseas opportunities. In this way, the private sector can play an active role in developing both scientific and administrative human resources.

Workshop participants agreed that international research centers should plan to play a larger role in transferring new technologies to developing countries. There should also be an increase in the funding provided through the centers.

The mandate of the centers should be critically evaluated to ensure that developing countries reap benefits when (and if) the private sector becomes a research and development partner. The centers should try to identify incentives for private sector involvement—including access to germplasm, marketable products, and human resources. They should identify activities in which the private sector can help with in-kind and technological contributions, and should encourage private sector representation on their boards. Some of the centers' personnel should have experience with and an

understanding of the private sector in order to facilitate contractual arrangements that may be established with multinational and national companies.

The workshop participants concluded with a discussion of the roles that should be played by international donor agencies (IDAs). Agencies distributing public funds should coordinate their donations in order to help rectify the current dilemma of overlapping funds. They should have a mechanism for matching funds contributed by the private sector. They should also award small "seed grants" as expeditiously as possible to support planning conferences designed to facilitate networking and collaboration.

IDAs and international centers should be attuned to and actively involved with national programs. They should pay close attention to what scientists and administrators in national programs have to say and provide guidance and assistance where needed. Some of the major areas that require recognition include: the need to develop national infrastructure; the need to develop a critical mass of people who are educated and trained in technology and management skills; and the need for facilities and equipment which will allow countries to benefit from the human resources they develop. Developing countries need more and better trained personnel for research efforts that employ both traditional and new methods for plant improvement andmanagement. USAID and other IDAs should provide training scholarships, recognizing that training needs vary from developing country to developing country.

In the context of the conference's private sector theme, particular note was made of the fact that developing country specialty crops, such as spices and fruits, and less-developed species of economic importance to a particular national economy, may be in need of development even though they may not be of interest to the private sector. The discussion was handicapped by the absence of representatives from established companies that use tissue culture (in vitro) technology. Only one company whose principal operation is micropropagation was represented at the conference.

Vaccine Development

Michael J. Burridge
University of Florida
Gainesville, Florida

Item 1: What collaborative opportunities or ideas have come to mind?

Dr. Muscoplat indicated that, while $2 billion are spent annually on animal health products in the United States (and $300-400 billion on human health products), only $50-60 million are spent in the United States on animal vaccines. From these data, he inferred that commercial companies were unlikely to allocate much research for vaccine development.

Dr. Doyle stated that ILRAD spent $6 million a year on research towards the development of a vaccine for East Coast fever. ILRAD has searched for a suitable industrial partner for development of this vaccine. It has moved its attention from the newly formed biotechnology companies who, because of dependence on venture capital, are unlikely to make a commitment to vaccines for tropical animal diseases with relatively low capital returns, to the established major pharmaceutical firms. Dr. Doyle believed that bilateral aid would have to fund vaccine development for the first 5-10 years before industry would be willing to become involved financially.

Discussions ensued on regulations relating to genetically engineered vaccines. Dr. Doyle pointed out that while these regulations must ensure satisfactory safety of the product, they must not be too stringent to prohibit testing of potential vaccines. Dr. Chema indicated that Kenya will require a certificate to show that the product to be tested is used in the country of origin. Dr. Shibley stated that USDA policy will require each new vaccine to be individually tested, with any vaccinia-virus-vectored vaccines limited to those strains of vaccinia virus used in the United States. He stressed that safety is the most important factor to USDA in considering authorization for testing of a vaccine.

It was pointed out that the resources in lesser developed countries (LDCs) for testing of genetically engineered vaccines are scarce, increasing the cost of vaccine development. Consequently, wherever possible, most research on recombinant DNA vaccine development will rely on existing facilities in the United States and Europe.

Item 2: What role is there for international donor agencies?

Dr. Muscoplat suggested that the most important role for the international donor agencies was to fund the basic research necessary to initiate development of genetically engineered vaccines. Dr. Doyle saw two additional roles for international donor agencies: (1) stimulation of involvement of universities in the basic research through grants or cooperative agreements; and (2) reduction of competition by fostering of collaboration between donor agencies.

There was discussion of training in biotechnology. While it was realized that international donor agencies had an important role in funding of biotechnology training for LDCs, there was a concern that most graduates in biotechnology will stay in developed countries due to the lack of career opportunities for them in LDCs outside the International Centers.

Item 3: What are the priorities for new initiatives in these areas?

Dr. Muscoplat indicated that the priorities were improved diagnostic tests for animal diseases and the development of genetically engineered vaccines that would eliminate the need for a cold chain. Dr. Doyle believed that the main priority was for a commitment to sustained funding for research on vaccine development since he believed that the development process will take up to 20 years.

Discussion from the floor emphasized two priorities, both related to funding for research on vaccine development. Firstly, it was stressed that funding for such research in LDCs must be competitive, with proposals subject to peer review, if the promises of modern biotechnology are to be realized and if the social constraints are to be overcome. Secondly, it was requested that efforts be made to attempt to gain collaboration between the donor agencies, perhaps by means of a donor forum.

Summary of Workshop

The objectives of this workshop, as set out in the conference program, could not be attained due to the lack of representatives from commercial industry. However, the workshop did emphasize the following points:

1. International donor agencies will have to provide the majority of funds for basic research into development of genetically engineered animal vaccines.

2. Most testing of genetically engineered vaccines will have to rely on facilities in the United States and Europe, especially during the early stages of development.

3. Universities in the developed world should conduct much of the basic research on development of genetically engineered vaccines through grants from, or cooperative agreements with, international donor agencies.

4. Competition in development of genetically engineered vaccines should be reduced by fostering collaboration between the international donor agencies.

5. Funding for the development of a genetically engineered vaccine must involve at least a 10-year funding commitment and must involve a competitive process with peer review of research proposals.

REGISTRATION LIST

Philip H. Abelson
Science Advisor
American Assoc. for Adv. of Science
1333 H St., NW
Washington, DC 20005 USA

Radha M. Acharya
Dep. Dir. General (AS)
Indian Council of Ag. Research (ICAR)
Krishi Bhawan
New Delhi 110001 India

Reid Adler
Spec. Consultant (AaL)
Amer. Assoc. for Adv. of Science
1333 H St., NW
Washington, DC 20005 USA

Vishwanath Prasad Agrawal
Director
RLAbB
P.O. Box 2128
Kathmandu, Nepal

Stuart S. Ainsworth
Manager, Gl. Ag. Projects
Abbott Labs—Ag. Products Group
14th & Sheridan Road
North Chicago, IL 60064 USA

Daniel Aldrich
Chancellor Emeritus and Chair
A.I.D. Research Advisory Committee
University of California-Irvine
Irvine, CA 92717 USA

Abdul Ally
Dir., DNA Chemistry
Biotechnica International
85 Bolton Street
Cambridge, MA 02140 USA

Carlos E. Ampuero
Sr. Specialist in Agriculture
Inter-American Development Bank
1300 New York Avenue
Washington, DC 20577 USA

Alva A. App
Sr. Sci. Adv.
Div. for Global Progress, UNDP
Rm. FF-1284One United Nations Plaza
New York, NY 10017 USA

Dil S. Athwal
Sr. Vice Pres., Programs
Winrock Intl. Inst. for Ag. Development
Route 3
Morrilton, AR 72210 USA

Russell Backus
Ag. Dev. Officer
A.I.D./AFR/TR/ARD
Rm. 2941 N.S.
U.S. Dept. of State
Washington, DC 20523 USA

Richard A. Baldwin
Consultant
Intl. Fund for Agricultural Research
4854 Thomas Avenue South
Minneapolis, MN 55410 USA

Rose A. Bannigan
Senior Program Officer
National Academy of Sciences
2101 Constitution Ave.
Washington, DC 20418 USA

Randolph Barker
Prof. of Ag. Economics
349 Warren Hall
Cornell University
Ithaca, NY 14853 USA

Castro Barreto
CENARGEN
Caixa Postal 10.2372
Brasilia, Brasil

John H. Barton
Prof., Stanford Law
Partner, International Tech. Management
1340 Harwalt Drive
Los Altos, CA 94022 USA

David D. Bathrick
Director of S&T/AGR
A.I.D./S&T/AGR
SA-18, Rm. 409
Washington, DC 20523 USA

Roger N. Beachy
Professor
Department of Biology
Box 1137
Washington University
St. Louis, MO 63130 USA

Doug Beck
ICARDA
P.O. Box 5466
Aleppo, Syria

Jean C. Bergaust
Special Assistant
A.I.D./SAA
SA-18, Rm. 522C
Washington, DC 20523 USA

Michel Bernon
Scientific Attache
French Embassy
410 Reservoir Rd.
Washington, DC USA

Robert B. Bertram
CGIAR Staff
A.I.D./S&T/AGR/CGIAR
Washington, DC 20523 USA

Sakarindr Bhumiratana
Dep. Director
Natl. Ctr. for Genet. Eng. &
Biotech.
Thailand

Charles S. Blankenstein
Consultant
S&T/FA
204 N. Spring St.
Falls Church, VA 22046 USA

Frederick A. Bliss
Professor
Department of Horticulture
1575 Linden Drive
University of Wisconsin
Madison, WI 53706 USA

Andrea Y. Blumberg
Agriculture Development Officer
A.I.D./ANE/TR/ARD
Rm. 4400 N.S.
Washington, DC 20523 USA

Norman E. Borlaug
Dist. Professor of Intl. Agriculture
Soil and Crop Sciences Dept.
Texas A&M University
College Station, TX 77843 USA

Nyle C. Brady
Senior Assistant Administrator for
Science and Technology
A.I.D.
Washington, DC 20523 USA

Sharma Ved Brat
President
Braton Biotech, Inc.
1 Taft Court
Rockville, MD 20850 USA

Lenie Breeze
Asst. Dir.
Biotechnology Institute
One Progress Blvd., Box 26
University of Florida
Alachua, FL 32615 USA

Roger Breeze
Director
USDA/ARS
Plum Island Anim. Disease Ctr.
P.O. Box 848
Greenport, NY 11944 USA

Mainor Briceno
Ag. Economist
University of Costa Rica
P.O. Box 35 Morivia
San Jose, Costa Rica

Peter Brumby
Livestock Development Advisor
World Bank
1818 H St., NW
Washington, DC 20433 USA

Gerald M. Bueing
Professor
Dept. of Veterinary Microbiology
103A Connaway Hall
Columbia, MO 65211 USA

Michael J. Burridge
Prof. and Chairman
Department of Infectious Diseases
Box J-137, JHMHSC/IFAS
College of Vet. Med., Univ. of Florida
Gainesville, FL 32610-0137 USA

Billy E. Caldwell
Head, Dept. of Crop Science
North Carolina State University
Box 7620
Raleigh, NC 27695-7620 USA

Cindy J. Callahan
Tech. Info. Specialist
USDA/APHIS/PPQ/BASS
Rm. 628A
Federal Building Library
Hyattsville, MD 20782 USA

Jerry Callis
Sr. Scientific Adv.
Plum Island Animal Disease Center
P.O. Box 848
Greenland, Long Island, NY 11944 USA

Peter S. Carlson
Vice President for R&D
Crop Genetics International
7170 Standard Drive
Hanover, MD 21076 USA

Patricia J. Carmack
Technology Reimbursement Reports
Suite One
5550 Friendship Blvd.
Chevy Chase, MD 20815 USA

Carolina A. Carter
Program Associate
Amer. Assoc. for Advancement of Science
1333 H Street, NW
Washington, DC 20005 USA

Roberto J. Castro
Ag. Economist
A.I.D./S&T/AGR/EPSA
SA-18, Rm. 403
Washington, DC 20523 USA

Nit Chantramonklasr
Plan. & Eval. Office
STDB
Bangkok, Thailand

Christopher G.D. Chapman
IBPGR/FAO
1001 22nd St., NW
Suite 300
Washington, DC 20437 USA

Uthai Charanasri
Dir. of Research
Bangkok Flowers Center Co., Ltd.
34/19 Petvasem Road
Nongkam
Bangkok 10160 Thailand

Chalermlarp Chauiprasit
Dir. of Research
Boon Rawd Brewery and Co., Ltd.
Bangkok 10300 Thailand

Sam Chem
Chief Vet. Res. Officer
Kenya Agriculture Research Institute
P.O. Box 57811
Nairobi, Kenya

Neville P. Clark
Dir., TX Ag. Res.
Texas A&M University
Experiment Station
College Station, TX 77843 USA

W. Ronnie Coffman
Prof. and Chairman
Dept. of Plant Breeding & Biometry
252 Emerson Hall
Cornell University
Ithaca, NY 14853-1902 USA

Rita Colwell
Director, Maryland Biotech. Institute
Microbiology Building
University of Maryland
College Park, MD 20742 USA

Ralph W. Cummings, Jr.
Ag. Economist
A.I.D./S&T/FA
SA-18, Rm. 311
Washington, DC 20523 USA

Dana G. Dalrymple
Ag. Economist
A.I.D./S&T/AGR
SA-18, Rm. 513
Washington, DC 20523 USA

Peter Day
Director, Center for Ag. Mol. Biology
Cook College, Rutgers University
Martin Hall, Rm. 101 A
P.O. Box 231
New Brunswick, NJ 08903 USA

Edmond A.L. Delanghe
INIBAP
Avenue Du Val De Montferrand
P.O. Box 5035
Montpellier Cedex 34032 France

Xavier Delannay
Res. Group Leader, App. Gen.
Monsanto Company
700 Chesterfield Village Parkway
St. Louis, MO 63198 USA

Robert J. Dellere
Head, Tech. Div.
Tech. Ctr. for Ag. and Rural Cooperation
Convention of Lome
Postbus 380-6700 AJ Wageningen
The Netherlands

H. Peter Delp
Science & Tech. Officer
ANE/TR/ENR
A.I.D.
Washington, DC 20523 USA

Johannes M.J. DeWet
Director, Cereals Program
ICRISAT
Patancheru P.O.
Andhra Pradesh 502 324 India

Haryanto Dhanutirto
Ministry of State for Res. & Tech.
JL. Veteran III
Jakarta PUSAT
Jakarta, Indonesia

John Dodds
Tissue Culture Specialist
Centro Internacional de la Papa
Apartado 5969
Lima, Peru

John J. Doyle
Dir. of Research
Intl. Lab. for Res. on Animal Disease
P.O. Box 30709
Nairobi 592311 Kenya

Raymond H. Dull
Dir., Bus. Dev.
Experience, Inc.
1200 Second Avenue South
Minneapolis, MN 55403 USA

Donald N. Duvick
Sr. Vice Pres., Res.
Pioneer Hi-Bred International
700 Capital Square
400 Locust St.
Des Moines, IA 50309 USA

Charles J. Eby
Dir., Biotechnology
Hill and Knowlton
901 31st St., NW
Washington, DC 20007-3838 USA

David L. Edwards
Western Biotechnology
13824 Recuerdo Drive
Del Mar, CA 92014 USA

Sam Engelstad
110 Central Street
Apt. 32
Wellesley, MA 02181 USA

Rolando Estrada
Universidad Nacional La Molina
Apartado 456
Lima, Peru

Diego F. Estrella
Chf., Tis. Cult. Lab.
Instituto Nac. de Invest.
Agropecuarias
Apartado 340
Quito, Ecuador

Luis H. Estrella
Dpto. en Ing. Genet. de Plant.
Unidad Irapuato
Apartado Postal 629
36500 Irapuato
Guanajuato Mexico

David A. Evans
Vice Pres., Corp. Research
DNA Plant Technology Corp.
2611 Branch Pike
Cinnaminson, NJ 08077 USA

Curtis Farrar
Exec. Sec.
CGIAR Secretariat
World Bank, Room N5045
1818 H St., NW
Washington, DC 20433 USA

D.C. Fauquet
ORSTOM
B.P.V. 51
Abidjan, Ivory Coast

Fernando Fernendez
Fundacion Honduran De Invest.
Agricola
Apartado Postal 2067
San Pedro Sula, Honduras

Malcolm J. Finlayson
Group Leader, Biochemistry
Mycogen Corporation
5451 Oberlin Drive
San Diego, CA 92121 USA

Kenneth S. Fischer
Deputy Director General, Research
Intl. Inst. of Tropical Agriculture
Oyo Road
PMB 5320
Ibadan, Nigeria

Lloyd R. Frederick
A.I.D./S&T/AGR/RNR
SA-18, Rm. 406F
Washington, DC 20523 USA

Maurice Fried
Consultant
FAO
130 Aspen Road
Fort Republic, MD 20676 USA

Emile A.G. Frison
Plant Pathologist
IBPGR
c/o FAO
Via Delle Terme Di Caracalla
Rome 00100 Italy

John F. Fulkerson
Prin. Scientist
USDA/CSRS
J.S.M. Bldg., Rm. 212
Washington, DC 20251 USA

William R. Furtick
Agency Director for Food and
Agriculture
S&T/FA
SA-18, Rm. 513
A.I.D.
Washington, DC 20523 USA

Jorge Galindo
Dir. of Res.
Plant Pathology Center
Colegio de Post Graduados
Chapingo, Mexico

Carl M. Gallegos
Chief Forester
A.I.D./S&T/FENR
SA-18, Rm. 503E
Washington, DC 20523 USA

Oluf L. Gamborg
Tissue Culture for Crops Project
Biology Dept.
Colorado State Univ.
Fort Collins, CO 80523 USA

John F. Gerber
Director, Biotech Inst.
Univers ity of Florida
One Progress Blvd.Box 26
Alachua, FL 32615 USA

Tejpal S. Gill
Chief
A.I.D./S&T/AGR/RNR
SA-18, Rm. 408
Washington, DC 20523 USA

James W. Glosser
Administrator
USDA/APHIS
Admin. Bldg., Rm. 312-E
Washington, DC 20250 USA

Robert Goodman
Vice President for R&D
Calgeme, Inc.
1920 Fifth St.
Davis, CA 95616 USA

E. Gordon Grau
Assoc. Prof., Endoc./Aquacult.
Hawaii Institute of Marine Biology
University of Hawaii
P.O. Box 1346
Kaneohe, HI USA

Peter Greening
Executive Director
IFAR
Suite 600, Rosslyn Plaza
1611 N. Kent St.
Arlington, VA 22209 USA

Peter Gregory
Director of Research
CIP
Apartado 5969
Lima, Peru

Peter C. Hall
Chief Oper. Officer
Resources Development Foundation
1920 N St., NW
Suite 400
Washington, DC 20036 USA

Pranee Hamelink
Lecturer
Dept. of Plant Pathology
Kasetsart University
Bangkok, Thailand

Patrick M. Hayes
Barley Plant Breeder
Cereal Research Project
Oregon State University
Corvallis, OR 97331 USA

John F. Hebblethwaite
Bus. Dev. Director
Monsanto Company
700 Chesterfield Village Parkway
St. Louis, MO 63198 USA

Robert W. Herdt
Director, Ag. Sciences
The Rockefeller Foundation
1133 Avenue of the Americas
New York, NY 10036 USA

M. Heuver
Boards for Plant Breeders Rights
P.O. Box 104
6700 AC
Wageningen, The Netherlands

Anne K. Hollander
Associate
The Conservation Foundation
1250 24th St., NW
Suite 500
Washington, DC 20037 USA

William A. Hoskins
Vice President of Marketing
Sungene Technologies Corp.
2050 Concourse Drive
San Jose, CA 95131 USA

Paul S. Hughes
Director, Corp. Rel.
World Resources Institute
1735 New York Avenue
Suite 400
Washington, DC 20006 USA

David Hutton
Hutton Associates
65 Old Land Road
Cheshire, CO 06410 USA

Curtis R. Jackson
Director
A.I.D./S&T/RUR
SA-18
Washington, DC 20523 USA

Robert D. Jackson
Dir., FERRO
USDA/OICD
American Emb./New Delhi
Washington, DC 20520-9000 USA

Norm Jacobs
President
Biotechnica International
85 Bolton Street
Cambridge, MA 02140 USA

Hari K. Jain
Dep. Director General
ISNAR
Oranje Buitensingel 6
P.O. Box 93375, 20509 A3
The Hague, The Netherlands

W. Clive James
Deputy Director General, Research
CIMMYT
Apdo. Postal 6-641
06600 Mexico, D.F.
51595 Mexico

Emil Javier
Sr. Res. Fellow
ISNAR
P.O. Box 93375
2509 AJ
The Hague, The Netherlands

Jesse M. Jaynes
Asst. Prof., Biochemistry
Dept. of Biochemistry
322 Choppin Hall
Louisiana State University
Baton Rouge, LA 70803 USA

Dorothy S. Jessop
Technical Info. Specialist
USDA/OICD
McGregor Bldg., Rm. 540
Washington, DC 20250 USA

David C. Jewell
Maize Breeder
CIMMYT Dept. of Agron. & Plant Genetics
411 Borlaug Hall, Univ. of Minnesota
1991 Buford Circle
St. Paul, MN 55108 USA

William F. Johnson
Intl. Res. Prog. Officer
BIFAD Staff
Rm. 5316 N.S.
U.S. Dept. of State
Washington, DC 20854 USA

Kerri-Ann Jones
Biotech. Adv.
USAID/New Delhi (ID)
U.S. Dept. of State
Washington, DC 20520-9000 USA

Monty P. Jones
Breeder
WARDA Reg. Mgr., Swmp. Rice Res. Stn.
PMB 678
Freetown, Sierra Leone

Quentin Jones
Dir., Latin Amer. Maize Proj.
BARC West
USDA/ARS
Bldg. 005, Rm. 117
Beltsville, MD 20705 USA

Peter J. Joyce
Asst. Researcher
Dept. of Horticulture
University of Wisconsin
1575 Linden Avenue
Madison, WI 53703 USA

Abbas M. Kesseba
Coordinator
International Fund for Ag. Development
Via del Serafico, 200
Rome, Italy

Rita Khanna
Staff Scientist
Braton Biotech, Inc.
1 Taft Court
Suite 101
Rockville, MD 20850 USA

G.S. Khush
International Rice Research Institute
P.O. Box 933
Manila, Philippines

William H. Klausmeier
Dir., Proj. Dev. Fund
Resources Development Foundation
1920 N Street, NW
Suite 400
Washington, DC 20036 USA

Philip C. Kosch
Assoc. Dean for Res.
College of Veterinary Medicine
Box J-125, HSC
University of Florida
Gainesville, FL 32610 USA

Stanley Krugman
Director, TMR
USDA/Forest Service
Washington, DC 20250 USA

George Kuo
Plant Physiologist
Asian Vegetable Res. & Development Ctr.
Tainan 74199 Taiwan

R. Laing
CIAT
Apartado Aereo 6713
Cali, Colombia

Klaus Lampe
International Rice Research Institute
P.O. Box 933
Manila, Philippines

Richard K. Lankow
Dir. of Prod. Dev.
Agri-Diagnostics Associates
2611 Branch Pike
Cinnaminson, NJ 08077 USA

Harold A. LaSieur
Sr. Comm. Adv.
A.I.D./S&T/AGR
SA-18, Rm. 406H
Washington, DC 20523 USA

Hei Leung
International Rice Research Institute
P.O. Box 933
Manila, Philippines

Mario Lobo
CIAT
Apartado Aereo
Cali, Colombia

Daniel E. Lounberg
Management Consultant
Arthur Young IMCG
3000 K St., NW
Washington, DC 20007 USA

Pamela Love
Program Analyst
USDA/CSRS
Rm. 213 JSM
14th & Independence Ave., SW
Washington, DC 20251 USA

Khaled M. Makkouk
Plant Virologist
ICARDA
P.O. Box 5466
Allepo, Syria

Calvin L. Martin
Asst. Dir. for Res.
A.I.D./AFR/TR
Rm. 2494
U.S. Dept. of State
Washington, DC 20523 USA

Alison McCusker
Head of Research
IBPGR/FAO/UN
Via Delle Terme Di Caracalla
Rome 00100 Italy

Connor F. McGrath
Consultant
Winrock International
11709 Bunker Hill Ct.
Union Bridge, MD 21791 USA

Terry L. Medley
Dir., Bio. & Env. Coord. Staff
U.S. Dept. of Agriculture/APHIS
6505 Belcrest Road
Federal Bldg., Rm. 406
Hyattsville, MD 20782 USA

Ronald Meeusen
Director of Plant Biotechnology Research
Zoecon Research Institute
975 California Avenue
Palo Alto, CA 94304-1104 USA

Jan C. Meneley
Vice Pres., R&D
Evans Biocontrol Inc.
895 Interlocken Parkway
Unit A
Broomfield, CO 80020 USA

Frank E. Mertens
Agronomist
A.I.D./S&T/AGR
SA-18
Washington, DC 20523 USA

Ellen Messer
Assoc. Prof.
Alan S. Feinstein, World Hunger Program
Brown University
Box 1831
Providence, RI 02912 USA

Henry I. Miller
Food and Drug Administration
Room 14-90
5600 Fishers Lane
Rockville, MD 20857 USA

Subhafh Minocha
Prof., Botany & Genetics
Dept. of Botany & Plant Pathology
University of New Hampshire
Durham, NH 03824 USA

J. Philip Moss
Prin. Cytogenetic.
ICRISAT
Patancheru 502 324 AP
Hyderabad 224016 India

Abdul Mujeeb-Kazi
Head, Wheat-Wide Cros.
CIMMYT
Lisboa 27
Apdo. 06600 Mexico, D.F.
Texcoco City, Mexico

Harthon I. Munson
Spec. Assistant
PVT Enterprise Bureau
USAID
Washington, DC 20523 USA

Charles Murphy
Natl. Prog. Staff, ARS
U.S. Dept. of Agriculture
Beltsville Ag. Research Center
Bldg. 005, Rm. 239
Beltsville, MD 20705 USA

Michael G. Murray
Prin. Res. Scientist
Agrigenetics Advanced Science Co.
5649 E. Buckeye Road
Madison, WI 53716 USA

Charles C. Muscoplat
Exec. V.P., New Business
Molecular Genetics Inc.
10320 Bren Road East
Minnetonka, MN 55343 USA

J. Nabney
Overseas Dev. Natural Resources Inst.
127 Clerkenwell Road
London WC 1X8LU
London, United Kingdom

Murray W. Nabors
Proj. Dir., Tissue Cult. for Crop Plants
Dept. of Botany
Colorado State University
Fort Collins, CO 80523 USA

Richard A. Neal
Sr. Fisheries Spec.
A.I.D./S&T/AGR/RNR
Washington, DC 20523 USA

John T. Neilson
Dir., Biotech. & Asst. Dean
1041 McCarthy Hall
University of Florida
Gainesville, FL 32611 USA

C.L. Niblett
Professor
Plant Pathology Dept.
University of Florida
Gainesville, FL 32611 USA

John Nickel
Director General
Intl. Center for Tropical Agriculture
Apartado Aereo 6713
Cali, Colombia

Ban L. Oei
Professor
Institut Teknologi Bandunt
Jalan Ganesya 10
Bandut, Indonesia

Peter Oram
Intl. Food Policy Research Institute
1776 Massachusetts Avenue, NW
Washington, DC 20036 USA

Wellington A. Otieno
Intl. Ctr. for Insect Physiol. & Ecology
Nairobi, Kenya

Elizabeth Owens
Biotechnica International
85 Bolton Street
Cambridge, MA 02140 USA

Marcia L. Packer
Writer/Editor
A.I.D./S&T/PO
SA-18
Washington, DC 20523 USA

William G. Padolina
Director
Natl. Inst. of Biotech. & Appl. Microb.
University of the Philippines at Los Banos
College, Laguna Philippines

Charles A. Panton
Tech. Asst. Spec.
USDA/OICD/TAD/WW
McGregor Bldg., Rm. 264
2121 K St., NW
Washington, DC 20250 USA

Rajendra Singh Paroda
Dep. Dir. General
Indian Council of Agriculture Research
Krishi Bhawan
Dr. Rajendra Prasad Road
New Delhi 1100011 India

Robert J. Parra
Managing Director
Intl. Biotechnology Group, Inc.
Suite 106-277
4200 Wisconsin Avenue, NW
Washington, DC 20016 USA

Ernesto Paterniani
Department of Genetics—ESALQ
P.O. Box 83
13400 Piracicaba
Sao Paulo, Brazil

Suresh S. Patil
Director, Biotech. Program
University of Hawaii
3050 Maile Way
Gilmore 410
Honolulu, HI 96822 USA

Gabrielle Persley
Scientist
ISNAR
Oranje Buitensingel 6
2511 VE
The Hague, The Netherlands

Kurt J. Peters
Deputy Director General
ILCA
P.O. Box 5689
Addis Ababa, Ethiopia

Mary L. Petrie
Prog. Anal./Doc. Ctrl.
USDA/APHIS/PPQ/BASS/Biotech.
Permit Unit, Rm. 630
6505 Belcrest Road
Hyattsville, MD 20782 USA

Ronald L. Phillips
Professor
Dept. of Agronomy and Plant Genetics
University of Minnesota
411 Borlaug Hall
St. Paul, MN 55108 USA

John A. Pino
Research Fellow
National Academy of Science
2101 Constitution Ave., NW
Washington, DC 20418 USA

Robert L. Plaisted
Prof. of Plant Breeding
Dept. of Plant Breeding & Biometry
252 Emerson Hall
Cornell University
Ithaca, NY 14853 USA

Donald L. Plucknett
Scien. Adv., CGIAR
World Bank
Rm. N5053
1818 H Street, NW
Washington, DC 20433 USA

Paiton Pomana
Director
Ag. Development Research Ctr. for NE
Thailand

Paitoon Ponsana
Ag. Development Res. Ctr. in Northeast
ADRC Khon Kaen Province
Thailand

Arthur L. Pope
Prof., Animal Sci.
University of Wisconsin
1466 Animal Sciences Bldg.
1675 Observatory Drive
Madison, WI 53706 USA

Hugh L. Popenoe
Director
Center for Tropical Agriculture
3028 McCarthy Hall
University of Florida
Gainesville, FL 32611 USA

Cal Qualset
Professor
Genetic Resources Conservation Program
University of California-Davis
258 Hunt Hall
Davis, CA 95616 USA

Raghav Ram
Sungene Technologies Corp.
2050 Concourse Drive
San Jose, CA 95131 USA

Dolores A. Ramirez
Geneticist
University of the Philippines at Los Banos
Los Banos, Philippines

P.S. Rao
Head, Plant Biot. Sect.
Bhabha Atomic Research Center
Trombay
Bombay 400 085 India

Ned S. Raun
Regional Rep.
Washington—Winrock International
1611 North Kent Street
Suite 600
Arlington, VA 22209 USA

Miloslav Rechcigl
Res. Review Director
Office of Science Advisor, SA-18
A.I.D.
Washington, DC 20523 USA

Richard A. Record
Ag. Sec. Spec.
U.S. Peace Corps
806 Connecticut Avenue, NW
Suite 701
Washington, DC 20526 USA

Janet A. Rice
AAAS Fellow
A.I.D./ANE/TR/ENR
Washington, DC 20523 USA

Thomas Rice
Executive Vice President
De Kalb-Pfizer Genetics
3100 Sycamore Road
De Kalb, IL 60115 USA

Max Rives
Tech. Consultant
CGIAR Secretariat
World Bank
1818 H Street, NW
Washington, DC 20433 USA

A. Ian Robertson
Dept. of Crop Science
University of Zimbabwe
P.O. Bag M.P. 167
Harare, Zimbabwe

William M. Roca
Head, Biotechnology Research Unit
CIAT
Apartado Aereo 6713
Cali, Colombia

James W. Rowe
Dir., West. Hemisph. Coop.
Amer. Assoc. for Advancement of Science
1333 H Street, NW
Washington, DC 20005 USA

J. Neil Rutger
Res. Leader
USDA/ARS
Agronomy Dept.
University of California
Davis, CA 95616 USA

Luis F. Salazar
Virologist
CIP
P.O. Box 5969
Lima, Peru

David C. Sands
Assoc. Professor
Dept. of Plant Pathology
Montana State University
Bozeman, MT 59717 USA

Margaret J. Sarles
Ag. Res. Specialist
A.I.D./LAC/DR/RD
Rm. 2242 N.S.
Washington, DC 20523 USA

Setijati Sastrapradja
Center for Research in Biotechnology
Jalan Raya Juanda 18
Bogor 16122 Indonesia

Richard L. Sawyer
Director General
Centro Internacional de la Papa
Apartado 5969
Lima, Peru

Stanley D. Schlosser
Foley, Lardner, Schwartz et al.
1800 Diagonal Road
Suite 510
Alexandria, VA 22313 USA

Thomas G. Schongalle
ISS Ltd.
1250 4th Street, SW
Suite 101W
Washington, DC 20024 USA

Karel R. Schubert
Director
Ctr. for the Conserv. of Plant Gen. Res.
Missouri Botanical Garden/Wash. Univ.
P.O. Box 299
St. Louis, MO 63166 USA

Loren L. Schulze
Assoc. Officer
A.I.D./ST/AGR
SA-18, Rm. 409
Washington, DC 20523 USA

J. Schweitzer
Environ. Specialist
A.I.D./PPC/PDPR/RP
Washington, DC 20523 USA

William R. Scowcroft
Dr./V.P., R&D
Biotechnica Canada, Inc.
170, 6815—8th St., NE
Calgary, Alberta T2E 7H[illegible] Canada

David E. Shaw
President
AgriTech Systems, Inc.
100 Fore Street
Portland, ME 04101 USA

George P. Shibley
Microbiologist
USDA/APHIS/VS
Federal Bldg., Rm. 838
Hyattsville, MD 20782 USA

Fernando A. Silva
157 Del Mar
San Francisco, CA 94117 USA

Mary Slocum
Native Plants, Inc.
417 Wakara Way
Salt Lake City, UT 84108 USA

Virgil Smail
Biotechnica International
85 Bolton Street
Cambridge, MA 02140 USA

Sumin Smutkupt
Coordinator
Bioscience/Biotechnology Program, STDB
Bangkok, Thailand

Maro R. Sondahl
Research Director
DNA Plant Technology Corporation
2611 Branch Pike
Cinnaminson, NJ 08077 USA

Frances Spivy-Weber
Dir., Intl. Prog.
National Audubon Society
801 Pennsylvania Avenue, SE
#301
Washington, DC 20003 USA

M. Allen Stevens
Vice President for Research
Campbell Soup Company
Campbell Place
Camden, NJ 08101 USA

B.A. Stewart
Conservation & Prod. Research Laboratory
USDA/ARS
P.O. Drawer 10
Bushland, TX 79012 USA

Lawrence Stifel
Director General
IITA/IBADAN, Nigeria
c/o Ms. M. Larkin, L.W. Lambourn & Co.
Carolyn House, 26 Dingwall Road
Croydon CR9 3EE United Kingdom

Deborah G. Strauss
Editor
Diversity
727 8th Street, SE
Washington, DC 20003 USA

Michael S. Strauss
Assoc. Proj. Dir.
Board on Agriculture
National Academy of Science
2101 Constitution Ave., NW
Washington, DC 20418 USA

Marjorie Sun
Res. News, Science
American Association for Adv. of Science
1333 H Street, NW
Washington, DC 20005 USA

Malee Suwanna-Adth
Head, Dept. of Microbiology
Kasetsart University
Thailand

M.S. Swaminathan
B4/142 Safdarjang Enclave
New Delhi 110029 India

John T. Sykes
Ag. Specialist
CIDA
200 Prom DV Portage
Hull Quebec, Canada

J.S. Tahardi
Plant Biotech. Lab.
Research Institute for Estate Crops
Jalan Paman Kencana #1
Bogor, Indonesia

Muhammad Tahir
Cereal Breeder, Cereal Program
Intl. Center for Ag. Research (ICARDA)
P.O. Box 5466
Aleppo, Syria

William H. Tallent
Assistant Administrator, ARS
U.S. Dept. of Agriculture
358-A Administration Bldg.
Washington, DC 20250 USA

James F. Tammen
Res. Sci. Dir. Ind. Rel.
Institute of Food and Ag. Sciences
University of Florida
1 Progress Boulevard, Mailbox 17
Alachua, FL 32615 USA

Rodrigo Tarte
Director General
CATIE
Turrialba, Costa Rica

Alva Taulananda
Group Vice Pres. for Dev.
Charen Pokphamo Co., Ltd.
Bangkok, Thailand

Claude Teisson
CIRAD
BP 5035
Ar Vol de Montferrand
Montpellier 34032 France

Joseph Thomas
Head, Biotech. Div.
Southern Petrochemical Ind. Corp., Inc.
Mount View
110 Mount Road
Guindy, Madras 600 032 India

Thalerng Tnamrong
Director
Science & Technology Development Board
Rajda Road
Bangkok, Thailand

Gary H. Toenniessen
Assoc. Dir., Ag. Sci.
The Rockefeller Foundation
1133 Avenue of the Americas
New York, NY 10036 USA

Sue A. Tolin
Prof. of Plant Path.
Lab for Molecular Bio. & Plant Stress
Glade Road Research Center
V.P.I. and State University
Blacksburg, VA 24061-0330 USA

Lamarr B. Trott
Sr. Fisheries Adv.
A.I.D./S&T/AGR
Washington, DC 20523 USA

Javier Trujillo
Inv. Doc.
Colegio De Postgraduados
Institucion de Ensenanza E
Investigacion en Ciencias Agricolas
Chapingo, Mexico

Joyce Turk
Livestock Specialist
S&T/AGR/AP
SA-18, Rm. 420G
Agency for International Development
Washington, DC 20523 USA

Bruce L. Umminger
Sr. Adv., Health Policy
Office of Intl. Health Policy
DES/IMP, Rm. 4329A, U.S. State Dept.
2201 C Street, NW
Washington, DC 20520 USA

Robert P. Upchurch
Assoc. Dean
College of Agriculture
University of Arizona
Tucson, AZ 85725 USA

Indra K. Vasil
Grad. Res. Prof.
511 Bartram Hall West
University of Florida
Gainesville, FL 32611 USA

Victor Villalobos
Prog. Head, Tropical Crop Improvement
CATIE
P.O. Box 15
Turrialba 56-02-32 Costa Rica

Ruxton H. Villet
Natl. Prog. Leader
USDA/ARS Natl. Program Staff
Bldg. 005, Rm. 232
BARC-West
Beltsville, MD 20705 USA

Cynthia K. Wagner
Business Analyst
Pioneer Hi-Bred International
2880 Grand Avenue, #1
Des Moines, IA 50309 USA

Ben Waite
A.I.D./S&T/AGR/RNR
SA-18, Rm. 420
Washington, DC 20523 USA

James L. Walker
Asst. Adm., IRAD
USDA/OICD
McGregor Bldg., Rm. 362
Washington, DC 20250-4300 USA

Dayuan Wang
DNA Plant Technology Corporation
2611 Branch Pike
Cinnaminson, NJ 08077 USA

David West
Agrigenetics Advanced Science Company
5649 East Buckeye Road
Madison, WI 53716 USA

Steven J. Wickler
Asst. Professor
Dept. of Animal Science
Cal Poly University
Pomona, CA 91768 USA

Samuel C. Wiggins
Prin. Horticult.
USDA/CSRS
Morrill Bldg., Rm. 217
Washington, DC 20251 USA

Floyd J. Williams
Winrock International
1611 North Kent Street
Arlington, VA 22209 USA

J. Trevor Williams
Director General
Board for Plant Genetic Research (IBPGR)
FAO/United Nations
Via Delle Terme Di Caracalla
Rome 00100 Italy

Erwin F. Workman, Jr.
Vice Pres., R&D/Operations
AgriTech Systems, Inc.
100 Fore Street
Portland, ME 04101 USA

Tilahun Yilma
Prof. of Virology
Veterinary Microbiology/
Immunology
University of California-Davis
Davis, CA 95616 USA

Fan Yunliu
Dir., Biotech. Ctr.
Chinese Academy of Agricultural
Sciences
30 Baishiqiao Lu
Beijing, Xijiao People's Rep. China

Yongyuth Yuthavong
Prof. of Biochem.
Dept. of Biochem.
Faculty of Science
Mahidol University
Rama 6 Road
Bangkok Thailand

Milton Zaitlin
Assoc. Dir.
Dept. of Plant Pathology
334 Plant Science Bldg.
Cornell University
Ithaca, NY 14853 USA

Francisco J. Zapata
Tis. Cult. Specialist
International Rice Research Institute
P.O. Box 933
Manila, Philippines