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PLANT BIOTECHNOLOGY
RESEARCH
FOR
DEVELOPING COUNTRIES

REPORT OF A PANEL
OF THE
BOARD ON SCIENCE AND TECHNOLOGY
FOR INTERNATIONAL DEVELOPMENT

NATIONAL RESEARCH COUNCIL

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NOTICE: The project that is the subject of this report was approved by the Governing Board of the National Research Council, whose members are drawn from the councils of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine. The members of the committee responsible for the report were chosen for their special competence and with regard for appropriate balance.

This report has been reviewed by a group other than the authors according to procedures approved by a Report Review Committee consisting of members of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine.

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This report has been prepared by an ad hoc advisory panel of the Advisory Committee on Technology Innovation, Board on Science and Technology for International Development, Office of International Affairs, National Research Council. Funding was provided by the Office of Research and University Relations, Bureau for Science and Technology, Agency for International Development, under Contract No. DAN-5052-C-00-6037-00.

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Office of International Affairs
National Research Council
2101 Constitution Avenue, N.W.
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Preface

At the request of the Agency for International Development's Office of Agriculture, the Board on Science and Technology for International Development (BOSTID) of the National Research Council (NRC) convened an NRC-appointed panel and a group of experts for two days of discussions concerning priorities in plant biotechnology research that could benefit agriculture in developing countries in the relatively near future—3–5 years. Funding was provided by AID's Office of Research and University Relations.

Plant biotechnology research has made great progress in recent years, and investment in it by the industrial countries is reflected in commercial products that are now beginning to be supplied to farmers and foresters. These include disease-free clones of fruit and vegetable crops, fast-growing trees for reforestation, biopesticides, and insect-resistant and herbicide-tolerant cultivars. As yet, relatively little research of this kind has focused on tropical crops beyond the Tissue Culture for Crops Project at Colorado State University supported by AID, the comprehensive biotechnology program on rice supported by the Rockefeller Foundation, and embryonic efforts on cassava. The objective, therefore, was to identify areas of biotechnology that, in the panel's view, held sufficient promise such that they could be promoted in AID client countries through new collaborative initiatives with U.S. scientific counterparts. Levels of funding that might be necessary, and possible time to achieve results, were also to be indicated.

Michael Dow of the BOSTID staff, Joel I. Cohen of AID's Bureau for Science and Technology, and I drew up an agenda for a two-day meeting and identified approximately thirty participants from academia, government, and industry, including experts from developing countries and the International Agricultural Research Centres.

The meeting was held on September 22 and 23, 1989, at the National Academy of Sciences' Georgetown Facility. This report consists of a number of parts: an executive summary, which is a synopsis of the rationale and the principal recommendations, and a report of the

workshop discussions, including a summary of the main issues surrounding each priority.

This report does not purport to be a comprehensive description of plant biotechnology nor a rigorous approach to setting priorities, and should not be confused with the broad analytical policy studies more typical of NRC committees. Rather, it conveys the sense of the panel on what activities would be "good bets" for AID support, for consideration by AID's Research Advisory Committee.

As with all endeavors that attempt both to bring together many different perspectives and distill large amounts of technical information into a coherent form accessible to the nonspecialist, based on only two days of discussions, a number of challenges were faced in the design and implementation of this effort. Therefore, a number of people deserve special thanks: the panelists, for their participation and helpful comments on the draft report; Robert Burris, Peter Carlson, and Ralph Hardy, who reviewed the draft report on behalf of the NRC Report Review Committee; a number of subject specialists who were unable to attend the meeting but who reviewed and commented on the draft report, especially David Evans, Calvin Qualset, and Steven Tanksley; and Joel Cohen, for his able technical liaison at S&T AGR and substantive assistance.

BOSTID staff and I appreciate all the assistance we received in preparing the report; we have tried to accommodate a great diversity of views as faithfully as possible. However, the nature of priority setting is to identify some activities for greater attention than others. Not all participants would necessarily agree with the emphasis in the final statement of recommendations, although none has registered specific objections. The responsibility for any shortcomings is entirely ours.

Robert W. Herdt
Chairman

April, 1990

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Promise of Biotechnologies for Africa

The direct use of biotechnology for plant propagation and breeding could dramatically raise crop productivity and overall food production in developing countries. Tissue culture techniques are creating more drought- and disease-resistant varieties of cassava, oil palms, and groundnuts. Plant genetic engineering may also result in coffee beans with less caffeine, in response to new consumer preferences, or faster growing tree species, which make reforestation easier. Better fermentation techniques in solid media, such as protein-enriched cassava flour, improve the nutritional value of crops. Embryo transfer may raise the reproductive capacity of livestock. Genetically engineered vaccines may overcome trypanosomiasis, thus opening up tsetse-infested grazing areas. Medical research on monoclonal antibodies, presently the fastest growing branch of biotechnology, is expected to result in more accurate medical tests and diagnostics. New vaccines against killer diseases are being developed. And integrated bioenergy systems may simultaneously generate food, animal feed, and fuel through microbial conversion of biomass.

The commercial use of new bioindustrial products may result in dramatically different patterns of agricultural production and trade. This may pose a threat to Africa's export crops. Laboratory-produced vanilla may soon put the livelihood of 70,000 vanilla bean farmers in Madagascar in doubt. And it is not unthinkable that consumers will soon have a choice between Kenya AA and biocoffee beans made in the United States. Another concern involves the privatization of research results. The current practice of patenting first-generation biotechnology products to cover any further use of bioengineered material will severely limit future competition. For developing countries this may also entail high licensing fees for seeds, which will make it harder to disseminate new crop varieties to smallholders. The widespread distribution of new bioengineered plant material may decrease the genetic diversity and may make crops increasingly vulnerable to new diseases.

A flexible African response to these competitive dynamics must be based on a close monitoring of biotechnological trends, more joint research and development partnerships with Western companies, and the development of substitute products. At the same time Africa will need dramatic improvements in its science education and agricultural training.

Sub-Saharan Africa: from crisis to sustainable growth
The International Bank for Reconstruction and Development

Executive Summary

INTRODUCTION

Plant biotechnology is a set of techniques designed to assist our abilities to change the genetic make-up of plants. They can be used to overcome disease, pest, and environmental constraints on production and to improve the quality of food and fiber crops. In so doing, these techniques, used in conjunction with conventional breeding programs, could make dramatic contributions to sustainable agriculture by producing improved crops that are more compatible with their environment. When assessing the usefulness of the new techniques to agriculture, it is critical to identify problems that have been difficult to solve with conventional approaches. AID's support for biotechnology should focus on the agricultural problems, and the products and processes needed to solve them, rather than on the technology itself. Cell and molecular biologists are the new partners of plant and animal breeders, agronomists, and pathologists. These new partnerships must be created to ensure integration of the new techniques into agricultural research and development programs, and to demonstrate their principles and applications in developing country agriculture.

PRIORITIES

I Institutional Priorities

Several key aspects of biotechnology research and development are institutional, rather than technical. The Panel recommends AID initiatives in three areas:

1. **Biosafety:** AID should assist developing countries to implement appropriate biosafety regulations. In addition to the legal, ethical, and environmental need for care when releasing genetically engineered organisms, there is an urgent practical need for development of procedures for field-testing of transgenic plants (and microorganisms), and the movement of these plants from country to country. Developing countries should be helped to formulate standards to fit their own needs, which

could be based on those in the United States rather than starting from scratch.

2. Intellectual Property: AID should facilitate international co-operation among U.S. research organizations, donor agencies, the International Agricultural Research Centres (IARCs), and LDC governments to make proprietary techniques, plasmids, and germplasm available to developing countries in a timely manner.
3. Training and Networking: AID should enhance biotechnology capabilities in LDCs through doctoral and postdoctoral fellowships and non-degree training for LDC plant biotechnologists. Networks of scientists in developing countries, linked to counterparts in the IARCs and industrial countries, should also be supported, to counter problems of isolation and of adequate access to scientific literature.

II Tissue Culture, Micropropagation, and Transformation

1. Tissue Culture: AID should continue to support the building of developing country capacity in plant tissue culture technologies that can augment conventional plant improvement programs, including micropropagation, cell selection, embryo rescue and haploid techniques, and regeneration. These techniques can support production of plants with increased tolerance to plant diseases, insect pests, and soil stresses, and they provide the foundation for more advanced biotechnology applications.
2. Micropropagation: AID should assist developing countries to acquire the capacity to use micropropagation to produce virus-free planting material of well-adapted forest, plantation, fruit, vegetable, and tuber crops. Though micropropagation is well-established for some crops, for others it is still in its early stages. Plants must be readily produced in the millions needed if farmers are to benefit from the technique.
3. Crop transformation: AID should support the development of transformation and regeneration techniques for cassava, millet, sorghum, and other major crops. The introduction of new genes through genetic engineering offers great potential for crop improvement, and has been demonstrated with rice, potato, tomato, soybeans, *Phaseolus* beans, and a number of other crops. Little or no work is currently directed at transformation of many tropical crops important to developing countries.

III Plant Disease and Pest Control

1. **Bt Strain Identification:** AID should assist developing countries to identify and clone Bt strains effective against major insect pests in tropical areas. *Bacillus thuringiensis* (Bt) bacteria produce a protein crystal that is selectively lethal to certain insects but not to others or to animals or humans. While there are many companies working on this worldwide, few of their efforts are focusing on tropical pests.
2. **Anti-Viral Strategies:** AID should support research to develop anti-viral strategies for combating plant viruses that attack *Phaseolus* beans, cassava, sweet potatoes, groundnuts, and tropical fruits and vegetables. The success of virus-protected potato, tomato, and tobacco plants should provide a strong foundation to expand research on this control approach for tropical crops.
3. **Pathogen Diagnostics-Probes:** AID should support research to develop DNA probes, as well as anti-sera and monoclonal antibody probes for plant bacteria, fungi, and viruses that attack crops of importance in the developing world. Sensitive and reliable tests are critical for the movement of germplasm to assure that seed is certifiably disease-free from key pathogens and for identifying diseases in the field. Kits for farmers could replace the need to culture and identify pathogens.

IV Genetic Mapping of Tropical Crops

1. **Genetic Mapping with RFLPs:** AID should assist CGIAR and developing country crop breeders to acquire the capacity to use RFLP maps wherever available, in plant breeding of rice, maize, sorghum, cowpea, and other crops.

It is not possible to establish a list of universal priorities for agricultural biotechnology, nor was this the essential charge to the panel. However, the opportunities and demand for work in biotechnology are so numerous, and available AID resources so limited, that funding should be focused on relatively few activities for maximum impact. The research activities recommended above are those that appear to present the greatest chance of ensuring that applications of biotechnology contribute to agricultural research in the developing world.

The order of presentation does not indicate their degree of importance in any particular country, as this will depend on national needs for agricultural research. Nevertheless, they are deliberately ordered so

as to reflect, in descending order, 1) the degree of general panel support for the areas, 2) increasing scale of complexity and resources required, and 3) types of research capacity that are prerequisite to achieving results with more complex techniques. They present research opportunities that AID should examine and fund selectively, after appropriate consultation with USAID missions, local governments, and the International Agricultural Research Centres (IARCs). Estimates of likely costs, and of the time required to achieve results in each area, are given in the detailed discussions that follow.

Proceedings

This section of the report summarizes the remarks of AID officials who addressed the meeting at its outset, and describes the process followed in responding to their charge: to produce an ordered list of priorities, and justification, in plant biotechnology research that can benefit developing country agriculture within a relatively short period, perhaps 3–5 years.

INTRODUCTORY REMARKS

William Furtick, Agency Director for Agriculture, AID

Dr. Furtick pointed out that the United States has been the leader of the global scientific community in developing scientific and commercial applications of biotechnology, but that of late, because of the local interests of states and federal agencies, American agricultural scientists have come to be less well-connected to the global system. The International Food Policy Research Institute (IFPRI) has been tracking global science related to agriculture, and people in agricultural research in the United States will be surprised to learn that they are part of a global system they are not plugged into. IFPRI estimates that 92–93 percent of the world's agricultural scientists reside outside the United States. Therefore, he said, we need to rethink our role. We were professors of many of those scientists; we have contributed to a sophisticated agricultural research system, made up of national, U.S. industrial, nonindustrial, regional, and multinational research centers—a big network. The new actor is the private sector. There is thus a need to examine the role of developing S&T systems for the future U.S. contribution to global science. We have to reach out to both public- and private-sector scientists. How to bring them together? Here the role of the IARCs is to serve as a focal point to bring the actors together. They are concentrated in LDCs, and donors find it profitable to tie into them. Currently, U.S. links are few and informal in relation to our financial contribution. The United States has the least formal relationship for cooperation among its scientists and

colleagues in the IARCs of any industrial country, and this needs to be rectified in our own self-interest. AID comes into these issues always "one priority behind," because of politics, and is seeking for guidance with the next phase of its biotechnology research support activities. Its emphasis is still on sustainability of agriculture and protection of the environment, using low-input technology. Plant biotechnology is most promising to support sustainability, particularly through disease and pest control, economic benefits of seed technology, low use of pesticides through plant-pest resistance, and diagnostics for identifying pathogens.

Both the Consultative Group on International Agricultural Research (CGIAR) system and AID focus on food crops, whereas farmers have a mix of food and cash crops. Increasingly, nonfood, nonfeed uses of food crops are underpinning the industrial world—20 percent of the U.S. corn crop goes to nonfood and feed uses; soybeans are used to make many products, including such things as printing ink. As the price of petroleum increases, as it is bound to, the use of developing countries' agricultural feedstocks in industry will grow. This is the global challenge for agriculture and biotechnology, and the context in which AID is looking for guidance. The commercial sector will continue to be the main producer of biotechnology, and AID wants to use its limited resources to develop a partnership with the private sector in developing technology for developing countries. Agricultural R&D supported by AID should have a 10-year payoff, within a 20-year perspective for public sector R&D.

**Joel I. Cohen, Biotechnology and Genetic Resource Specialist,
Office of Agriculture, AID**

Dr. Cohen summarized current S&T/AGR programs in support of plant biotechnology R&D that are focused on integration of the technology with traditional breeding programs. These include the Tissue Culture for Crops Project (TCCP), which seeks to develop and transfer validated tissue and cell methodologies to developing countries. The improvement of tropical rhizobia through conventional and molecular manipulations is being undertaken by the Nitrogen Fixation by Tropical Agricultural Legumes (NIFTAL) project. Research approaches include:

- Harboring multiple copies of Nif structural genes and infection genes;
- Introducing host-range and symbiotic plasmids into rhizobia from germplasm collections for strain improvement; and

- Identifying and transferring genes that make certain strains super competitors to less competitive rhizobia associated with tropical legumes.

Biotechnology is being used for the development of new vaccines against livestock diseases prevalent in developing countries. A National Science Foundation (NSF) report commissioned by AID included the recommendation for the development of a recombinant vaccine for rinderpest, an acute, highly contagious viral disease of cattle capable of killing an estimated two million cattle a year. Development of a new vaccinia-vectored vaccine for rinderpest illustrates the advantages to be obtained from new technologies. The recombinant vaccine is thermostable and should be easier to produce. It was developed by scientists at the University of California, USDA-ARS, and California Biotechnology Incorporated, through a subcontract, an interesting example of private sector collaboration.

The control of two other hemoparasitic diseases, anaplasmosis and babesiosis, was also recommended by the NSF report. In this project, surface proteins providing protection are being identified, followed by cloning of the genes that express these proteins. Their effectiveness as protective immunogens will be tested in recombinant vaccinia constructs. Nucleic acid probes to detect subclinically infected animals, or carriers, are under development. They will be used for economic impact studies and to provide the ability to differentiate vaccinates from infected animals.

David D. Bathrick, Director, S&T/AGR, AID

Dr. Bathrick pointed out that the tissue culture project was controversial when it started: it was believed to be too sophisticated a technology for developing countries. Through the achievements to date, and particularly the integration of biotechnology with conventional breeding in the Cooperative Research Support Programs (CRSPs), the success of the approach has been demonstrated. Now what AID is looking for are not only research priorities but "new ways of doing business" through linkages with the IARCs and with private sector activities. Budgets are not increasing, so there must be careful attention to comparative advantages, trade-offs, and relative impact. The animal vaccine model demonstrated the comparative advantage of the animal biotechnology approach. Are there further areas ready for AID in plant biotechnology to be supported in the Office of Agriculture strategy?

CHARGE TO PANEL

In the ensuing discussion, panelists raised the issue of the level of biotechnology programs that AID might fund; if the United States is to tap into the global system more effectively, there must be adequate funds to make this possible. AID officials indicated that they expect about \$9 million to be available for the next five years, including programs of the Office of Agriculture and the Science Adviser's competitive grants Program in Science and Technology Cooperation (PSTC), although more funds could be found from the budgets of the USAID missions and from regional programs. Part of the Panel's responsibility, therefore, is to present clearly the priorities and their justification. It was agreed that the Panel would produce an ordered list of priorities, and that these would be put in a context of levels of funding at \$1 million and \$10 million per year, respectively. These priorities would be addressed to the broader development community, in the hope that they could influence national policy and contribute to the internationalization of agricultural science as well. However, the primary focus is on the benefit that plant biotechnology could bring to agriculture in developing countries. And this is urgent: the world is on the verge of another industrial explosion, and there will be need for agricultural feedstocks and for greater efficiency of production and transformation in agriculture.

Given the objective of persuading the donor community of the importance of supporting plant biotechnology research, it is critical to focus on possibilities for successes within a relatively short period—3–5 years. Research is also supported by USDA and NSF, which can complement AID in the basic sciences. AID support will also be key in strengthening human resource development in client countries. It was pointed out that the CRSPs have provided good examples of combining research, training, and support for national systems, as well as of the examples of cooperation with IARC's (which are also supported by AID). It was also pointed out that it takes a long time from research to commercialization, and it is important to target a few critical areas and stick with them.

METHODOLOGY

A set of materials was sent to the panel members in advance of the meeting, consisting of: 1) summary statements of the personal plant biotechnology priorities of a number of panelists and biotechnologists unable to attend the meeting, 2) recommendations from two prior

meetings, and 3) a number of background papers (see Appendix 4). Following the discussion of their charge (above), panelists commented on the current relevance of previous priorities.

Having agreed on the Panel's charge, participants then discussed the best way to focus on priorities. Some time was spent in discussing the possibility of categorizing priorities by areas, crops, or technologies. However, the panel was evenly divided with respect to interest among these three areas, so an alternative approach was devised: panelists were first asked to indicate their individual priorities for three activities in which spending roughly \$2 million a year would achieve the desired objectives; all the suggested activities were then ranked by the process described below; finally, duplicate activities were eliminated and a consolidated list developed.

The modified delphi technique employed began with each participant, in turn, proposing a specific biotechnology research project "to improve agriculture significantly in USAID client countries"; these 97 projects are listed in Appendix 2. Participants then ranked their top nine priorities, and similar or closely related activities among the other 89 were grouped with the top eight in groups of related projects. Panelists were then asked to indicate measures or indicators of success for each of the eight groups, estimate how long a particular project might take to be completed successfully, and how much of first \$1 million and then \$10 million per year in additional funds they would allocate to each of the groups to achieve successful results.

Based on the panelists' suggestions, the chairman and staff reduced the number of projects in each of the eight categories by eliminating duplicates, combining closely related ideas, identifying projects likely to stretch beyond the 3-5 year AID target, or those which did not fit within the S&T/AGR responsibility. The final list of activities was then circulated to panelists and those invited plant biotechnologists who were unable to participate, to obtain their comments and suggestions, which were incorporated into a final draft. (The results are briefly presented in the Executive Summary and reported below with more detailed discussion.) The report was then reviewed by a group other than the authors according to procedures approved by the NRC Report Review Committee and appropriately reflects their comments.

Conclusions and Recommendations

INTRODUCTION

Agricultural research has been a priority area of AID's programs from the outset. AID is a major contributor to the Consultative Group on International Agricultural Research (CGIAR) and to the Board on International Food and Agricultural Development (BIFAD) networks, in addition to its bilateral and regional programs with numerous developing countries. Why is it necessary now to consider additional allocations from AID's limited resources to strengthen this new area of agricultural research?

The answer lies in a combination of things: the development of scientific techniques that have dramatically increased the sophistication of our ability to manipulate biological material, and in which the United States has played a leading role, coupled with the fact that at the same time there exists a new generation of agricultural, health, natural resource management, and energy problems that afflict AID's client countries. Without a deliberate effort, it is unlikely that AID will be able to transfer those technologies to countries with which AID has traditionally had a special relationship, where technical assistance has been directed at measures to increase local economic self-sufficiency, and where national agencies have the ability to solve their own development problems. Within AID's current agricultural projects there are opportunities for supporting plant biotechnology activities, as there are within USAID mission-funded programs, as well as the Office of the Science Adviser's competitive research grants Program for Scientific and Technical Cooperation (PSTC). This report is designed to identify the activities that are likely to yield useful results within a feasible time frame for a range of AID client countries.

The sophisticated scientific techniques range from those that use personal computers to direct and monitor complex scientific processes to biological techniques that manipulate minute units of genetic material. These have made possible routine performance by technicians of complex biochemical transformations that would have been impossible by leading scientists a decade ago. They have also created the potential to produce disease- and pest-resistant cultivars of agricultural crops, the potential to reduce the time and cost required to multiply

elite specimens of shrubs and trees for widespread use, and the potential to identify and incorporate genes with useful properties into crops (thereby reducing the time involved in the hit-or-miss procedures of traditional breeding programs).

Biotechnology and Sustainable Agriculture

An emerging goal of international agricultural research is the integration of biotechnology with conventional crop improvement programs when these alone have not resolved specific productivity or environmental constraints. Diverse initiatives in biotechnology have been implemented to address constraints on developing country agriculture and to respond to requests from scientists eager to see new technologies applied to national agricultural priorities.

Many of these applications of biotechnology are components of agricultural research that will also contribute to understanding and implementing sustainable agricultural practices. The new tools of biotechnology, which present the ability to manipulate unrelated or distant genomes, can lead to the development of more environmentally compatible crop plants, which, in turn, increase productivity of the world's farmers. These genetically improved plants, developed in conjunction with scientists assessing their long-term impact on environmental integrity of soil and water resources, will become a major part of sustainable farming practices. If the concept of sustainability is to serve as a practical guide for agricultural research, then it must include the use of technologies that both enhance and sustain productivity through genetic, as well as soil and water, resources.

Thus, biotechnology should contribute directly to sustainable agriculture while leading to a reduction in the use of agrochemicals and providing for control of pests that have eluded present technologies. Incorporation of more environmentally compatible crops expressing new sources of tolerance for either abiotic or biotic stresses will enhance productivity and, thus, exert a profound effect on the developing economies.

New cultivars, derived through an integrated use of biotechnology, plant breeding, and agronomy will become part of sustainable agriculture because they reduce the use of pesticides through insect- and disease-tolerant transgenic plants. In fact, distribution of improved seed is recognized as one of the best mechanisms for technology transfer. Farmer income will also be increased by reducing input costs as crops become available, which makes more efficient use of nutrients.

Clearly, new initiatives are called for that enhance the contributions of biotechnology to sustainable agriculture. This need has been inde-

pendently recognized by several recent publications (National Research Council, 1989; Hauptli et al., 1990; Edwards et al., 1990; and Schneiderman and Carpenter, 1989).

These technologies are urgently needed by developing countries to ensure the improvement of tropical crops so that biological technologies can be substituted for chemical technology. Opportunities to improve storage and protein quality of tropical crops such as cassava could also be undertaken. The more productive that acreage can become in the developing world while being farmed in a sustainable manner, the greater the benefit to be derived. International donor support is required to ensure that the scientific and technical knowledge becomes available that will enable farmers in developing countries to meet demands placed upon them to sustain or increase their current levels of production.

Biotechnology, and the conservation of the genetic resource base upon which it depends, must play a direct role in the evolution of our understanding of sustainable agriculture. New applications of biotechnology already demonstrating greater precision in the manipulation of plant genomes must be applied as well to crops of importance to LDCs. Rather than divorce new genetic technologies from sustainable agricultural research, their active incorporation should be encouraged.

It should be emphasized, however, that very limited financial resources are available, either from LDCs themselves or through donor agencies, for many of these new initiatives. Therefore, priority objectives must be identified and targeted to overcome specific constraints of recognized national importance. Next, proposals should be submitted that address these constraints and these will then be peer-reviewed. Awards should be based on ability to provide new approaches to problems of primary importance when considered among other pressing needs for donor support.

The single most important decision is the target crop (and the object of its genetic improvement) to which research is applied. Biotechnology is highly crop specific. Techniques proven for one crop must be adapted for other crops, and because most crop biotechnology research is being conducted in industrialized countries, little research is focused on developing country crops. AID should, therefore, fund only work on crops of importance in the developing world.

It should be recognized that the techniques of plant biotechnology cannot replace traditional crop breeding programs. In fact, most of the activities of traditional plant breeding programs, especially those of field screening and wide-scale testing, must be effective for scientists to take advantage of biotechnology's promise. One critical step in assessing the usefulness of the new techniques is to identify problems

that have proved to be intractable to conventional approaches, and that may benefit from the application of new technologies. The focus in biotechnology thus needs to be on agricultural problems, and the products and processes needed to solve them, rather than on the technology itself. Cell and molecular biologists are the new partners of the plant and animal breeders, agronomists, and pathologists, not their replacements. These new partnerships are critical to ensure integration of the new techniques into existing agricultural research and development programs, and to demonstrate their principles and applications in developing country agriculture especially to meet the needs of the small subsistence farmer.

A brief discussion of how classical plant breeding and biotechnological approaches differ and complement each other is provided in an introduction to a companion NRC report: "Field Testing Genetically Modified Organisms" (included here as Appendix 3). Some plant biotechnologies are well suited to developing countries: because it is scale-neutral, biotechnology can be a mechanism to create new cottage industries in which a village-sized fermenter can produce a variety of products in support of local agriculture; they are labor-intensive in some aspects, and could provide employment in areas such as routine multiplication of elite laboratory strains of, for example, plantlets produced in a micropropagation facility for field use, or in testing numerous individuals for a specific gene; their application requires local biological resources and must be carried out, for the most part, in the countries where the crops are to be grown; they are relatively low cost because they do not require expensive scientific equipment; they are not sophisticated because they can be understood and used by many individuals with appropriate training. And they are being requested by developing country scientists. They are thus logical candidates for technical assistance programs.

On the other hand, they do require effectively functioning laboratories with water, electricity, glassware, chemicals, enzymes, and some sophisticated equipment. The costs of equipping such a laboratory are roughly comparable to the costs of training a scientist to the Ph.D level in molecular biology. A further complicating factor is that plant biotechnology products—the genetically engineered organisms or the very genes themselves—are increasingly being patented by private companies in the industrial world. Indeed, some observers believe that newly identified useful genes, and new varieties of organisms that contain them, will only be produced because they are patentable—since it is not worth the investment in the work by private companies if they cannot reap the benefit. This trend towards genetic research being sponsored by the private sector is being vigorously promoted

by the United States and most European governments. But, new genetically engineered varieties of most tropical crops (and other organisms) are unlikely to be research targets of U.S. and European companies, since the opportunities of recovering the costs of development through sales of the product would appear to be minimal.

Existing research networks in the tropics (including the CGIAR system of IARCs) are likely to be users rather than producers of these genetic materials since they have neither the expertise nor the organization to produce final products. Furthermore, most IARCs are located in countries that do not patent living organisms and hence have difficulty in negotiating with companies to acquire patented genetic material that might be used in developing countries. There is thus a clear need for a technical assistance program that encourages and assists developing country researchers and agencies to acquire the technologies and focus on critical problem areas of local importance.

Although AID and the scientists recognize that animal and microbial biotechnology also offer opportunities for developing countries, priorities for those activities are considered elsewhere.

PRIORITIES

Plant biotechnology is a methodology to change or assist in changing a plant's genetic make-up, which can be used to overcome disease, pest, and environmental constraints on production, or to improve the quality of food and fiber crops. Although plant biotechnology research and development has the potential to make dramatic contributions to global agriculture, it is likely to be most productive when used in conjunction with traditional breeding programs to supplement the system of crop improvement.

I Institutional Priorities

Several key aspects of biotechnology research and development in developing countries are institutional, rather than technical. The Panel recommends AID initiatives in three institutional areas:

1. **Biosafety:** AID should assist developing countries to design and implement appropriate biosafety regulations. Regulations are not yet clearly defined in many countries (they are still receiving detailed attention in the United States and Europe, for example). In addition to the legal and ethical need for care in the release of

genetically engineered organisms, there is an urgent practical need for development of procedures for field-testing of transgenic plants and microorganisms, and the movement of these plants and organisms from country to country. Developing countries could modify U.S. standards to fit their needs, rather than starting from scratch. But they need objective, authoritative advice. Many countries have difficulty in deciding which products to license and which companies to allow to develop and test products, and as a result, err on the side of caution, so that the use of safe products is not being permitted. This suggests an important role for AID's technical assistance through USAID missions and regional programs.

2. **Intellectual Property:** AID should take the lead in promoting the development of U.S. policy to promote international cooperation in intellectual property rights among U.S. research organizations (public and private), donor agencies, the IARCs, and LDC governments to make proprietary techniques, gene clones, and germplasm available to developing countries in a timely manner. Related issues beyond intellectual property rights involving foreign ownership in LDC companies, profit repatriation, and government licenses are also important factors influencing the private sector interest in engaging in biotechnology in developing countries. AID, as the U.S. government agency responsible for collaboration with developing country governments and the international and bilateral donor community, especially as regards agricultural research collaboration, is the appropriate agency to influence the evolution of this policy area.
3. **Training and Networking:** AID should enhance biotechnology capabilities in LDCs through doctoral and postdoctoral fellowships, and nondegree training for LDC plant biotechnologists, but should also continue appropriate research training in complementary agricultural sciences. Networks of scientists in developing countries, linked to counterparts in the IARCs and industrial countries through such mechanisms as periodic workshops, and, where feasible, electronic networks using FAX and personal computers/modems, should also be supported, perhaps through Cooperative Research Support Programs (CRSPs). This is an effective mechanism to counter problems of isolation of many LDC scientists, and the equally difficult problem of adequate

access to scientific literature. AID could assist national research laboratories in LDCs to gain access to U.S. biological databases.

Scientific Priorities

It became clear throughout the discussions that it was not possible to establish a list of universal priorities for agricultural biotechnology because of several factors: 1) the varied nature of agriculture around the globe, 2) the varying degree of technological competence among developing countries, 3) the different constraints on crop production at different locations, 4) the differences in crop importance, and 5) the differences in problems such as pests, disease, and drought, and the many types and stages of technology available.

The activities discussed below are likely to contribute to important crops affecting many people in developing countries and should ensure that integrated applications of biotechnology contribute to agricultural research in the developing world. However, the opportunities for work in biotechnology are so numerous, and AID's resources so limited, that funding should be focused on relatively few activities if it is to have any effect. The order of presentation does not indicate their degree of importance in any particular country, as this will depend on national needs for agricultural research. (Nevertheless, they are deliberately ordered so as to reflect, in descending order, 1) the degree of general panel support for the areas, 2) increasing scale of complexity and resources required, and 3) types of research capacity that are prerequisite to achieving results with more complex techniques).

To develop improved crop varieties in a 3- to 5-year time span, it is necessary to use "off-the-shelf" technologies (see recommendations II.1 and 2). The development of new research techniques such as the transformation-regeneration systems (II-3), insect and disease techniques (III-1, 2, and 3), and construction of RFLP-generated maps for crops where they do not now exist (IV-1), will require the recruitment of staffs, the training of individuals, the discovery of small changes in the technology necessary to apply it, and the subsequent application of the techniques to crop improvement. In general, 3-5 years is a reasonable time in which to expect scientific results, but these approaches will require closer to 10 years before they can be seen as having any significant impact on the development of new varieties.

AID is urged to concentrate its resources on a limited number of the recommended activities, after appropriate consultation with USAID missions, local governments, and the IARCs.

II Tissue Culture, Micropropagation, and Transformation

The crop plants of today had their origins in the fields of early farmers who selected plants with desirable traits and maintained cultivars to meet agricultural needs. Controlled matings (hybridization) of plants through the sexual process is the cornerstone of classical plant breeding. Spontaneous and mutagen-induced variation in plants has also produced a variety of genetic traits that have been used in plant breeding. Hybridization and selection of plants with new combinations of traits have been used to increase genetic diversity. By repeated hybridization and selection, new traits have been introduced into varieties already proven successful in agriculture. There are two major limitations that exist with classical plant breeding, however. The first is an extraordinarily large degree of variability from which a low frequency of desired plants must be identified. Second, the gene pool—the source of genes accessible to the breeder—is generally limited to the same or closely related species. Much of modern plant biotechnology is devoted to overcoming these two limitations, by speeding up the reproduction of “elite” plants of known desirable genetic characteristics, and by identifying useful genes and finding techniques (“genetic engineering”) to introduce them into plants that could not occur through classical breeding. Tissue culture, micropropagation, and plant transformation/regeneration are three related aspects of plant biotechnology.

Tissue culture has been practiced for thousands of years as a means of regenerating large numbers of whole plants (often perennials) from cuttings or “slips” as an alternative (usually faster and more certain) to propagation from seeds. Grafting is a subset, in which elite slips are grafted onto vigorous rootstock. Biotechnology has resulted in the development and use of hormones and nutrient media to enable recalcitrant species to be regenerated and otherwise improve tissue culture. Micropropagation is a type of tissue culture in which plant cells are cultured in the laboratory to multiply them and their constituent genes. The multiplied cells can then be used to produce literally millions of regenerated genetically identical “plantlets” for reforestation or for distribution to plant breeders or farmers. The cells can be transformed genetically, by introducing desired genes, prior to the multiplication stage. These related transformation techniques are central to modern plant biotechnology. Although capabilities with tissue culture have developed rapidly over the past 15 years, it is recognized that many developing countries still do not have effective capacities. Support should be supplied on a carefully selected basis to provide enhanced developing country capabilities in these technologies, particularly

micropropagation, because of their importance in producing virus-free stocks, and as a component of a broader program—that is, as an essential step in transformation. Their potential for creating new genetic combinations through somaclonal variation or tissue culture thus far has produced few, if any, crop varieties directly. Therefore, this particular area should be viewed with caution.

1. **Tissue Culture:** AID should continue to support the building of developing country capacity in basic plant tissue culture technologies, which are necessary for genetic engineering and can augment conventional plant improvement programs, including micropropagation, cell selection, embryo rescue, haploid techniques, protoplast fusion, and protoplast regeneration. These techniques may directly produce plants with increased tolerance to plant diseases, insect pests, and soil stresses, and, perhaps more important, they provide the foundation for more advanced biotechnology applications.

An effective program in this area would likely require, for each species addressed, a total of between \$200,000 and \$500,000 per year, and would likely require 3–5 years to produce routine methods of tissue culture. (A functioning laboratory requires around \$75,000 of capital equipment.)

2. **Micropropagation:** AID should assist developing countries to acquire the capacity to use micropropagation to produce virus-free planting material of forest, plantation, fruit, vegetable, and tuber crops. Though micropropagation is well-established for some crops, for others it is still in its early stages. Plants must be readily produced in the millions needed if farmers are to benefit from the technique. Some crops are recalcitrant, and there are problems of automation and quality control in the LDCs. This kind of R&D would be particularly suited for USAID mission support.

An effective program in this area would require \$50,000 per year per crop over 3–5 years to implement. Regional networking of researchers in these areas might be employed to enhance effectiveness. Up to \$1 million per year would produce an effective and focused program at an estimated average scale of \$50,000 per year per crop.

It should be recognized that large numbers of genetically identical plants, whether produced by traditional plant breeding or newer technologies, entail the potential hazard of vulnerability, so care must be exercised to guard against disease and insect pests.

3. **Crop Transformation:** AID should support the development of

transformation and regeneration techniques for tropical crops for which such techniques are not now available, such as cassava, taro, millet, sorghum, and groundnuts. Such transformation has been demonstrated with rice, potato, tomato, soybeans, *Phaseolus* beans, and a number of other crops, but increased efficiency is required to make it routine.

The ability to perform routine transformation in a crop is fundamental to the application of most of the exciting biotechnology approaches. Until routine transformation is achieved, the most productive and innovative techniques will have to wait. Therefore, this should receive high priority and be executed in a team effort that brings together molecular and plant scientists, breeders, and others. Despite promising results, only the Solanaceae and a few other plants can be transformed in high frequency and routinely. This deserves support for creative thinking among scientists who know plants and tropical agricultural problems as part of the team. After initial broad screening, the program should concentrate at most on the 2-3 crops where transformation has been demonstrated to be feasible, while modest efforts could continue on a broader range of crops.

It would probably require annual funding of from \$500,000 to \$1,000,000 to support transformation/regeneration of one crop in five years. Rice, for instance, has been the subject of transformation research costing about \$1 million a year over the past five years. Transformation of rice has been demonstrated by half-a-dozen laboratories. Wheat and millet should be accomplished within the next three years. Cassava has thus far been difficult to regenerate; most grain legumes are difficult, and maize and sorghum are very difficult. However, studies in this field undergo dramatic changes in a short period of time. Since the meeting, the first successful transformation of maize with production of fertile seed has been accomplished.

Examples of candidates for transformation and regeneration research, because of their special disease problems, include cassava in Latin America and Africa, legumes, especially cowpeas, in Africa and *Phaseolus* beans in Latin America, potatoes in Latin America and Asia, and yams in Africa. Anti-viral strategies await the ability to transform these plants to incorporate virus-resistance genes.

III Plant Disease and Pest Control

Among the potentially most useful genes occurring in plants (and microorganisms) are those that confer resistance to attacks on the

plants by other organisms—bacteria, viruses, fungi, insects, and weeds or parasitic plants. Traditional breeding programs have had considerable success in incorporating these qualities, but modern plant biotechnology offers the possibilities of greater specificity. Diagnostic/pathogen probes can identify the cause of plant attack with great specificity, and measures can then be employed to enhance resistance to viruses or other organisms responsible. Biotechnology has also made it possible to harness the natural protective mechanisms of microorganisms to produce “biopesticides” and also to transfer the genes responsible for such protection into plants to give them built-in protection.

Bacillus thuringiensis (Bt).

Among these possibilities, one of the most successful involves a bacterium, *Bacillus thuringiensis* (Bt). Bt, a naturally occurring, aerobic, soil-borne bacterium, produces protein crystalline inclusions during its sporulation cycle. These inclusions are insecticidal for many agronomically destructive insect pests, especially lepidoptera (moths). Recently, the host range for Bt activity has been extended to include certain members of the dipteran (fly) and coleopteran (beetle) insect families. Bt crystal preparations have been used as commercial insecticides for over 20 years. As such, Bt is one of the most widely used biologicals for insect pest control. Widespread use of Bt for insect control has been limited by the narrow host range of susceptible insects and its instability in crop fields, requiring repeated and costly applications.

The advent of molecular genetic engineering techniques offers great promise to proponents of biopesticide control. Numerous Bt toxin genes have been cloned and sequenced from several strains active against all three families of insect pests. Recent advances in Bt transformation techniques have facilitated the construction of recombinant strains with expanded insecticidal host ranges for use as commercially important insecticidal sprays. In addition, identification and cloning of these genes has led to their recent introduction into plants and the successful production of transgenic tomato and tobacco species with genetically engineered insect resistance. As transformation techniques are developed for a greater range of crops, the production of insect-resistant, transgenic cereals, fruits, and vegetables may also be possible. No information exists at present on the toxicity or safety of varieties containing Bt genes. However, there is a great deal of information on Bt itself, and many years of experience that should be

applicable to concerns regarding the safety of transgenic plants and the ability of insects to develop increased resistance to Bt.

These advances offer great potential benefits for the less developed countries. Widespread application of traditional chemical pesticides is accelerating the evolution of resistant insect species. The intensive use of chemicals, furthermore, may lead to serious health problems and the contamination of soil and groundwater by chemical pesticide residues, as in the United States and Europe. But, as novel Bt toxin genes are isolated, cloned, and utilized to produce new germplasm in the more industrial countries, the corresponding exercise of intellectual property rights may diminish Third World access to this beneficial technology. Unless developing countries establish increased capabilities in biotechnology research, the Bt strains most effective in their conditions may not be identified. Cooperation for mutual benefit should be the objective.

Third World countries offer a vast untapped market for both the use and development of novel Bt products. Conceivably, valuable strains of Bt that are effective against pests causing severe agricultural losses in the United States are indigenous to many of these developing countries. Thus, there is potential for joint ventures and transfer of technology between the private sector in industrialized countries and the LDCs that have developed some expertise in Bt research. Training in strain identification, gene isolation, recombinant strain production, bioassay procedures, and new fermentation technologies would make LDCs viable prospective partners for research and commercialization of Bt products and ultimately contribute to their self-sufficiency.

Anti-Viral Strategies by Production of Transgenic Plants.

The DNA coding for the Tobacco Mosaic Virus (TMV) has been transferred via a complex series of steps into "transgenic" tobacco plants that exhibit resistance to infection by the TMV. This work has been done by researchers at Monsanto and Washington University who also collaborated to produce resistance against cucumber mosaic virus and other viruses.

The indications thus far are that even though the virus particle shape and 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. This seems to represent a generic method to produce virus resistance in plants. The method may provide virus resistant material that can be given to plant breeders for introduction of the gene into existing 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 simplify enormously the work of plant breeders who generally search for resistance genes in other plant varieties, cultivars, or species, and spend years improving the agronomic characteristics of plants containing the gene.

The virus diseases that occur throughout the tropics severely reduce the yields of most vegetable and fruit crops. 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 virus protection. Although there are numerous target crops for application of this technology in developing countries, research to meet the targets will require international collaboration because of the degree of technical sophistication involved. Such research will be difficult 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. Nevertheless, selected university laboratories and international research centers are capable of carrying out the work if they can retain appropriately trained scientists, organize collaboration with industrial country scientists, and receive adequate financial and technical input from developed countries.

1. **Bt Strain Identification:** AID should assist developing countries to identify and clone Bt strains effective against major insect pests in tropical areas, in cooperation with advanced laboratories and private companies in the United States. While there are many companies working on this worldwide, few of their efforts focus on tropical pests.

The identification of effective Bt strains could probably be accomplished in 2–3 years at a cost of around \$50,000 per strain. The production of bioinsecticides based on effective strains collected in LDCs and tested against LDC pests is a longer and more expensive process. An operating program in this area would likely require between \$70,000 and \$300,000 for each Bt strain and would probably require 8 years or more to produce significant results with cloning, testing, and scale-up. Once cloned, Bt could be directly produced by microbial processes, or inserted into crop plants through transformation. Collaboration with private industry is essential. While focusing on identifying Bt strains, AID should encourage developing countries to develop integrated pest management techniques and programs, within

which Bt should be an important, though by no means the sole, approach.

Examples of priority pests identified by developing country panelists for which effective Bt strains could make a significant contribution to biocontrol are *Plutella xylostella* (diamondback moth), *Spodoptera exigua* (beet armyworm), *Chilo partellus* (stalk borer), and *Manuca testulalis* (bean pod borer).

2. Anti-Viral Strategies: AID should support research to develop strategies for combating plant viruses that attack major crops in the developing world, such as *Phaseolus* beans, cassava, sweet potatoes, groundnuts, and tropical fruits and vegetables. The successful demonstration of virus-protected potato, tomato, and tobacco plants illustrates the usefulness of this control approach.

An effective program in this area would likely require between \$150,000 and \$500,000 for each virus addressed and would require 2-5 years to produce significant results, depending on the target crop and virus problems. This field is developing rapidly and significant progress could be made in a short time on some crop plants, depending on the ability to transform the target crop with the appropriate DNA. That is, effective routine crop transformation must be achieved for the target crop before this approach can be productive.

Examples of priority virus diseases are: cassava viruses, and geminivirus on cowpeas and groundnut, in Africa; cassava viruses, and geminivirus in *Phaseolus* beans, in Latin America; soft rot in potato and other virus diseases of nutritionally important fruits and vegetables, and geminivirus in legumes in Asia.

3. Pathogen Diagnostics/Probes: AID should support research to develop DNA probes, as well as antisera and monoclonal antibody probes for plant bacteria, fungi, and viruses that attack crops of importance in the developing world. There are three different problems for different pathogens: 1) probes may be unknown; 2) methods of producing the sera or their use in identification of pathogens may be unreliable; or 3) sera may be available but their production is not scaled up. This vast area for research and development requires sensitive and reliable tests to assure that seed is certifiably disease-free from key pathogens before it can be moved. Field-usable kits are already available for some pathogens or mycotoxins in the United States, and if developed they could be used in Third World countries instead of present methods, which require workers to culture and identify pathogens.

An effective program in this area would likely cost between \$80,000

and \$150,000 per pathogen, and would probably require 4–5 years to produce reliable field-tested results.

There is some disagreement concerning the emphasis that should be given to develop these biotechnology tools. Some feel they are not really necessary, or practical for field use, since the diseases of major crops ought to be relatively simple to identify by experienced farmers, extension agents, or researchers. Others point out that environmental concerns are pushing for replacement of broad-spectrum chemical control with highly specific biological techniques, and the ability to discriminate precisely among pathogens is crucial to the effective use of biologicals.

Examples of the pathogens for which diagnostic probes would be especially useful are thus very location specific, depending on intimate understanding of the local ability to identify pathogens, the technical capacity available, and the relative importance that would be attached to diagnostics as opposed to other priorities. U.S. biotechnology companies receive many requests from developing countries for assays for viruses (especially from seed companies, and for cucumber mosaic virus and tomato spotted wilt) and fungal diseases (especially phytophthora and other root invaders) and mycotoxins, particularly aflatoxin. The cost of the assay or kit is apt to limit general application to high value commodities, such as cocoa, citrus, vanilla, and black pepper. This might be an area in which to encourage proposals for competitive grants under AID's PSTC biotechnology module.

IV Genetic Mapping of Tropical Crops

Linkage analysis of genetic traits has long been used by geneticists and plant breeders to "mark" plants that carry desirable genes. Application of molecular biology techniques have made available a greatly expanded set of markers, known as restriction fragment length polymorphisms (RFLPs). RFLPs are most useful to monitor traits that are difficult to screen, by following closely linked pieces of DNA through the breeding process. For crop breeding, RFLPs can assist in manipulating quantitative traits, pathogen and parental identification, plant propagation biology, and in other ways.

1. Genetic Mapping with RFLPs: AID should assist CGIAR and developing country crop breeders to acquire the capacity to use RFLP maps in plant breeding of rice, maize, sorghum, cowpeas, and other crops where these maps are becoming available.

Even though a number of RFLP crop maps are available, few developing country crop breeders have the equipment or training to

use them. About \$200,000 is needed to establish an RFLP laboratory that could be used for several crops in an LDC. Development of RFLP maps are highly crop specific and require considerable expertise. For example, it may require \$200,000 per year for 3 years to develop an RFLP map for a crop on which no start has yet been made (such as groundnuts). This work would have to be done at an advanced laboratory in the United States and linked to plant traits by a laboratory in a developing country able to grow the crop under field conditions and make agronomic observations. Material from a number of countries could be tested in this way without duplicating the rather sophisticated facilities required for this long-term work.

OTHER AREAS

There were many other high priority agricultural research activities identified by the Panel (Appendix I) that deserve continuing support, but lack a specific biotechnology approach. Among the most important are, *Striga* control, biological nitrogen fixation (BNF), and salt and drought tolerance.

Striga (witchweeds) may constitute the greatest biological constraint to cereal production in Africa, especially for crops under water and/or nutrient stress conditions. Breeding programs are currently under way to incorporate resistance into sorghum and cowpeas; there is no known source of resistance to *Striga* in millet or maize. If recent advances in selection of resistant cultivars lead to identification of resistant genes, this could provide the breakthrough necessary to employ biotechnology techniques in multiplying resistant strains of cereals with desirable local agronomic characteristics.

The genes responsible for nitrogen fixation in rhizobia have been identified, but they have not yet been successfully transferred to crops. Transferring Nif genes to plants is proving very complex, is already receiving research attention in industrial countries, and should not be encouraged in developing countries for the moment. Improving rhizobia for legumes may be a worthwhile target for developing countries.

The genes responsible for salt and drought tolerance appear to be numerous, and their practical employment is some way off. However, selection of salt-tolerant strains of plants for salinized areas by plant breeding may offer useful opportunities in a number of countries (see National Research Council, 1990, *Saline Agriculture: Salt-Tolerant Plants for Developing Countries*, National Academy Press, Washington, D.C.).

Appendix 1

Participants

- Robert W. Herdt, Director, Agricultural Sciences, Rockefeller Foundation, 1133 Avenue of the Americas, New York, NY 10036. *Chairman*
- John D. Axtell, USAID Sorghum/Millet Collaborative Research Support Program, Department of Agronomy, Purdue University, West Lafayette, IN 47907
- Roger N. Beachy, Professor, Department of Biology, Box 1137, Washington University, St. Louis, MO 63130
- Robert H. Burris, Department of Biochemistry, University of Wisconsin, Madison, WI 53708
- Larry Butler, USAID Sorghum/Millet Collaborative Research Support Program, Department of Biochemistry, School of Agriculture, Purdue University, West Lafayette, IN 47907
- John H. Dodds, Tissue Culture Specialist, Centro Internacional de la Papa, Apartado 5969, Lima, PERU
- Richard K. Lankow, Director of Product Development, Agri-Diagnostic Associates, 2611 Branch Pike, Cinnaminson, NJ 08077
- David MacKenzie, Director, National Biological Impact Assessment Program, Suite 330, Aerospace Building, U.S. Department of Agriculture, 901 D Street, S.W., Washington, DC 20251-2207
- Abdul Mujeeb-Kazi, Head, Wheat-Wide Cross Program, Centro Internacional de Mejoramiento de Maiz Y Trigo, Lisboa 27, Apdo Postal 6-641, Delegacion Cuauhtemoc 06660, Mexico City, MEXICO
- Murray W. Nabors, Director, USAID Tissue Culture for Crops Project, Department of Botany, Colorado State University, Fort Collins, CO 80523
- Wellington Otieno, Deputy Director, International Centre for Insect Physiology and Ecology, P.O. Box 30772, Nairobi, KENYA
- James Steadman, USAID Bean/Cowpea Collaborative Research Support Program Department of Plant Pathology, University of Nebraska, Lincoln, NE 68583
- Thira Sutabutra, Director, Research and Development Institute, Kasetsart University, Bangkhen, Bangkok, THAILAND
- Indra K. Vasil Graduate Research Professor, Department of Vegetable Crops, University of Florida, Gainesville, FL 32611
- J. Trevor Williams, International Board for Plant Genetic Resources, c/o International Fund for Agricultural Research (IFAR), Suite 600, 1611 North Kent Street, Arlington, VA 22209
- H. Martin Wilson, Cell Biologist, Garst Seed Co., Highway 210W, Box 500, Slater, IA 50244

AGENCY FOR INTERNATIONAL DEVELOPMENT

David D. Bathrick, Director, Office of Agriculture, Bureau for Science and Technology

Judith Chambers, AAAS Fellow, Office of Agriculture, Bureau for Science and Technology

Eugene R. Chiavaroli, Assistant Administrator, Bureau for Science and Technology

Joel I. Cohen, Biotechnology and Genetic Resource Specialist, Office of Agriculture, Bureau for Science and Technology

Vincent Cusumano, Office of Agriculture, Bureau for Science and Technology

William Furtick, Acting Agency Director, Food and Agriculture, Bureau for Science and Technology

Lance Jepson, Agriculture and Natural Resources, Office of Technical Resources, Bureau for Africa

Robert Schaffert, Office of Agriculture, Bureau for Science and Technology

NATIONAL RESEARCH COUNCIL STAFF

Michael Dow, Board on Science and Technology for International Development

Jay Davenport, Board on Science and Technology for International Development

Maurice Fried, Board on Science and Technology for International Development

John Pino, Board on Agriculture

Appendix 2

Initial List of Projects

Initial listing of plant biotechnology research projects that could be used to improve agriculture significantly in USAID client countries.

1. Identify Bt genes effective against insects with developed resistance to chemical pesticides
2. Use protoplast fusion between Kallar grass and wheat to initiate gene introgression to include salt tolerance.
3. Use of tissue culture to speed up the process of plant disease, insect pests, and drought resistance.
4. Micropropagation for virus elimination in mass cloning of forest, plantation, fruit, vegetable and tuber crops.
5. Initial stages of gene transfer work for insect and virus resistance.
6. Floret culture methodology to facilitate haploid propagation in wheat for enhancing breeding efficiency and alien gene introgression and molecular mapping.
7. Develop RFLP mapping in sorghum to follow *Striga* resistance.
8. Develop *Striga*-tolerant cultivars of sorghum/cowpea/millet.
9. Transformation/regeneration of *Musa* spp.
10. Increase horticulture crop micropropagation.
11. RFLP map of cowpea.
12. RFLP map of potato.
13. Characterize *Striga* signal receptor sites.
14. Develop antisera/monoclonal antibodies for specific bacteria, fungi and viruses.
15. Develop *in vitro* methods for introduction and storage of legume germplasm.
16. Develop aflatoxin-resistant maize.
17. Develop new sources of resistance through *in vitro* culture of sorghum and millet.
18. Use of Rhizobia for improved biological nitrogen fixation.
19. Produce somaclonal variants for insect pest resistance.
20. Production of bioinsecticides.

21. Embryo rescue for wide hybridization.
22. Generation of qualitative trait variation through tissue culture.
23. *In vitro* screening for biotic/abiotic conditions in cereals.
24. Develop quality-protein sorghum.
25. Develop abiotic stress cultivars of semi-arid condition-tolerant crops.
26. Develop anti-viral strategy for geminivirus and other viruses.
27. Assist LDCs to improve and monitor biosafety.
28. Develop conservation techniques for cassava.
29. Develop nonradiation tests for pathogen detection/identification.
30. Characterize and clone gene for *Striga* resistance.
31. Develop simple immunoassays for field use in LDCs.
32. Pathogen detection techniques for edible legumes.
33. Develop low-pollution herbicides.
34. Characterize and clone gene for *Striga* resistance in millet.
35. Use of haploids for recessive alleles for stress and disease tolerance.
36. Integration of novel genes for insecticidal properties of plants (especially sorghum).
37. Improve protein and oil quality/content by tissue culture selection and/or transformation.
38. Successful TB/IRV wheats: generate other callus-culture-induced transformation.
39. Transposon tagging of waxy blue gene.
40. Develop bird-resistant sorghum cultivars without tannins and antinutritional components.
41. Develop resistance to mealy bugs, whiteflies and thrips.
42. Develop repository or genetic constructs for international use.
43. RFLP map of groundnuts/peanuts.
44. Increase efficiency of antisera production through ideotypes.
45. Develop method to detect and quantify *Striga* seed in soil.
46. Regeneration system for *Phaseolus*.
47. Fund state-of-the-art workshop for LDC biotechnologists.
48. Develop use of e.g. pseudomonads for biocontrol.
49. Implement methods to determine full potential of somaclonal variants to obtain useful variation for disease/stress tolerance.
50. Develop resistance to cereal stemborers of sorghums/millet.
51. Begin program for LDC breeders to participate in product development.
52. Biochemical marker for identification of alien species markers into wheat.
53. Development of semi-arid cultivars with stress adaptation to marginal lands.
54. Develop transformation/regeneration of roots/tubers.

55. Transformation techniques for sorghum.
56. Develop method for field testing transgenic plants from LDC's.
57. Correlation of secondary metabolites (especially phenolics) with insect and disease resistance.
58. Identify biocontrol agent and localized production systems for cotton pests (lepidoptera).
59. RFLP map for *Phaseolus*.
60. BTG No.1 reference, cotton boll worm, beet armyworm, diamondback moth control.
61. Genetic mapping of neem isolates for insecticidal properties.
62. Develop methodology and protocol for bioassay of insect control via Bt in LDC's.
63. Isolation of genes for increasing specific resistances.
64. Enhance biotechnology capabilities in LDC's through postdoctoral fellowships.
65. Development and application of non-radiation probes for breeding diagnostics.
66. Develop aflatoxin-tolerant peanut/groundnut cultivars.
67. Papaya tissue culture transformation/regeneration for viral resistance.
68. Cassava enzyme system for postharvest physiological rotting.
69. RFLP map for millet.
70. Business managers in AID and IARC's to negotiate agreements with private biotechnology companies.
71. Develop geminivirus resistance in *Phaseolus*.
72. Increase plant/rhizobia BNF system capability.
73. Determine human resource base for doing biotechnology in LDC's.
74. Literature grants for LDC's.
75. Short/long and degree- training for LDC plant biotechnologists.
76. Application of transfer of cowpea virus resistance.
77. Wheat protoplast regeneration.
78. Develop approaches to limit cyanogenesis in cassava.
79. Mechanism to link public/IARC research to the private sector.
80. Transformation/regeneration of groundnut/peanut.
81. Establish crop/technology network for technology transfer/information sharing.
82. Develop standard ELISA format for all agricultural research centers working on the same crop.
83. Develop varieties of fast-growing trees/bamboo for high use/value potential.
84. Determine economic impact of AID investments in biotechnology in LDC's.
85. Somaclonal variants for efficient fertilizer use.

86. Characterization of fragrance genes in Basmati A.70 rice.
87. Transformation/regeneration for some cucurbits in LDC's.
88. Formal methods for setting priorities in biotechnology by crop and by country.
89. Integrating biotechnology laboratories with breeders to ensure use of new germplasm.
90. Develop role for tax incentives for investment in biotechnology in LDC's.
91. Increase attraction of biotechnology for LDC students.
92. Role of social scientists in increasing the value of biotechnology in LDC's.
93. Maximizing biological nitrogen fixation.
94. Increasing availability of information on funding sources, techniques, expertise, and literature on biotechnology.
95. Role of biotechnology in sustainable agriculture.
96. Tissue culture for trees.
97. Development of probes for *Frankia*/mycorrhizal fungi identification.
98. Can biotechnology extend the limits of crop management research?

Appendix 3

Field Testing Genetically Modified Organisms

The following section is excerpted from chapter 2 (Introduction) of *Field Testing Genetically Modified Organisms*, National Research Council, 1989, National Academy Press, Washington, D.C.

Recent advances in biology have proceeded at an astonishing rate, and biologists now have the means, by directly modifying genes, to alter living organisms more quickly and more precisely than has been done by nature and humans over millennia. There is general agreement that this ability can yield far-reaching improvements in our environment and in medical and agricultural practice. However, field testing of promising products of the new technology has been slowed by the absence of a full scientific consensus on the relative safety and risks of introducing modified organisms into the environment. Furthermore, the specific questions that are most important to consider in making decisions have not been agreed on. Hence, this NRC committee was formed to attempt to determine a reasoned consensus about what scientific questions must be asked and how such questions can aid in the development of a decision-making process based soundly on the facts of science.

The history of efforts to reach a common ground about the relative safety or hazard of genetic modification of organisms can be traced directly to the early 1970s, when advances in biological knowledge had given scientists the tools to recombine DNA in the laboratory into new sequences.

THE GENETIC MODIFICATION OF ORGANISMS: MERGING CLASSICAL AND MOLECULAR TECHNIQUES

This report describes the properties of plants, microorganisms and the environment that must be evaluated when the introduction of a genetically modified organism into the environment is being planned. In this introductory section we explore the basic biological principles that underlie both classical and molecular means of altering the genetic makeup of organisms and explain how our interpretation of these principles leads to the conclusion that the products of classical and molecular methods fundamentally are highly similar. Both methods of modifying DNA produce an organism (product) that is genetically different from the starting organism regardless of the method (process) used. The molecular techniques are often more precise than classical techniques and can modify single nucleotides of bacterial genomes. Molecular modifications surpass classical techniques in their ability to introduce a great variety of traits from a wide range of donor organisms into the recipient organisms. As a corollary, the molecular techniques can generate a greater range of phenotypes than the classical methods. These principles as they apply to plants and microorganisms are discussed in greater detail in the sections of this report dedicated to the two kinds of organisms.

Plants and microorganisms contain nucleotides in combinations and arrangements that endow the organisms with genetic determinants for many traits. Other regions of DNA may control the expression of the traits. The DNA provides the raw material upon which genetic modifications depend. The evolution of new forms of crop plants and microorganisms results from selecting organisms with desirable traits from populations that possess heritable variation. When genetic variants are selected to produce the next generation, the population is changed with respect to the frequency of individuals having the selected characteristic. In the terms used in population genetics, selective breeding or propagation changes gene frequencies, and the population differs in some aspect from its predecessor even though the change may be small.

Modification of microorganisms and plants can be performed by either "classical" or "molecular" methods. No hard line exists between the two categories, especially with microorganisms. For this report, we generally include as classical those means of genetically modifying

organisms that were used before recombinant DNA techniques were developed. One major distinction of classical methods is that they are relatively undirected modifications of the genome. Molecular methods provide more flexibility and control and thus are more specific in directing the modifications toward a planned end product.

Methodological and biological distinctions exist in culturing microorganisms and plants, but one feature of the new genetic technologies is that they permit us to manipulate plants at the cellular level. This technology provides new commonalities to plant and microbial breeding.

Classical methods are those in which the genetic recombinations occur essentially in a natural way; desirable offspring variants are then selected in the laboratory or the field. Examples include spontaneously mutating microorganisms and sexually cross-bred plants. The term classical also includes some methods called that only because they predate the introduction of modern gene-splicing techniques. The latter include such human-mediated techniques as exposure of organisms to chemical mutagens or physical agents such as x-rays and ultraviolet radiation. We also include as classical those mechanisms of DNA transfer that occur without chemical treatment of a cell's envelope, such as transformation, conjugation, and transduction in microorganisms.

Molecular methods of genetic modification include the newer methods for modifying DNA in which one nucleotide can be substituted for another at a predetermined site in a DNA molecule (site-directed mutagenesis). Molecular gene transfer methods are used for transfer of genetic material between donor and recipient cells that have diverged widely through evolution and probably do not exchange DNA without laboratory manipulation. However, it is important to recognize that certain gene transfers thought impossible in nature a few years ago because of the phylogenetic distance between donor and recipient have now been shown to occur in the laboratory and it is suggested they may occur in nature. For example, there is evidence that a gene or genes for erythromycin resistance was transferred between the gram-negative bacterium *Campylobacter* and unrelated gram-positive bacteria (Brisson-Noel et al., 1988). Recent laboratory experiments have accomplished gene transfer between *Escherichia coli* and streptomyces (Mazodier et al., 1989) or yeast (Heinemann and Sprague, 1989). Another example relates to the natural transfer of DNA from the bacterial species *Agrobacterium* to plant cells (Nester et al., 1984). Plasmid genes from this bacterium probably were transferred into a species of tobacco early in the evolution of the genus *Nicotiana*, and they became integrated into the plant chromosome. These genes, or

their remnants, have been detected in a variety of different species of *Nicotiana*, which presumably evolved from the original infected plant (Furner et al., 1986).

PLANT MODIFICATIONS—CLASSICAL TECHNIQUES

Spontaneous and mutagen-induced variation in plants has produced a great variety of genetic traits that may be used in plant breeding. The crop plants of today had their origins in the fields of early farmers who selected plants with desirable traits and perpetuated plants to meet agricultural needs.

Controlled matings (hybridization) of plants through the sexual process is the cornerstone of classical plant breeding. Hybridization and selection of plants with new combinations of traits have been used to increase genetic diversity. By repeated hybridization and selection, new traits could be introduced into varieties already proven successful in agriculture. Hybridization is often possible between species, usually within the same genus. However, many interspecific hybridizations require human-mediated intervention to facilitate the sexual process. For example, developing embryos are excised and cultured on nutrient media before being grown as plants in the field. The male or female fertility of such hybrids is often reduced so that they themselves must be hybridized with one of the parents or with a closely related species. Alternatively, fertility can be restored by doubling the chromosome number. With sexual hybridization, the resulting progeny contain full complements of genes from each parent. The challenge for plant breeders is to select for the genes which result in a plant's exhibiting the desired combination of traits. Because interspecific hybrids, and even many intraspecific hybrids, have a parent that may be poorly adapted to survive and grow in an agriculturally useful way, considerable effort is required to examine large numbers of plants to find the desired combinations of traits.

Two major limitations exist with classical plant breeding. The first is an extraordinarily large degree of variability from which a low frequency of desired plants must be identified. Second, the gene pool—the source of genes accessible to the breeder—is limited to those species which can be sexually hybridized.

PLANT MODIFICATIONS—MOLECULAR TECHNIQUES

In principle, any gene can now be introduced into any plant by one of several possible molecular modification techniques. At present, the

most frequently used agent for DNA transfer is the common soil bacterium *Agrobacterium* (Nester et al., 1984). This organism evolved a mechanism for transferring part of its plasmid into plant cells, where it is integrated randomly into the chromosome (Peerboone et al., 1986). The introduced DNA is inserted within this plasmid DNA as a "hitchhiker." Once integrated into the plant's chromosome, the DNA is transmitted from parent to offspring and follows the pattern of Mendelian inheritance. Virtually all dicotyledonous plants are amenable to transformation by *Agrobacterium*, but most monocotyledonous plants appear to be resistant.

A technique frequently used to transform monocotyledonous plants such as maize and rice, is electroporation: this technique requires removal of the plant cell walls before the DNA is added. These naked cells, or protoplasts, often do not synthesize new cell walls readily. Thus, regeneration of whole, fertile plants from protoplasts has limited use of molecular gene transfer, especially in cereal grasses. More recently, DNA-coated gold or tungsten particles have been "shot" into plant cells, and stable, genetically transformed plants have been regenerated from the cells or organized tissue (Klein et al., 1987). This technique may be suitable for introducing DNA into plant chloroplasts (Boynton et al., 1988) and mitochondria (Johnston et al., 1988), as well as into the plant nucleus. Current research is directed toward introducing DNA into specific plant tissues that have the greatest probability of regenerating genetically modified plants.

COMPARISON OF CLASSICAL AND MOLECULAR TECHNIQUES IN PLANTS

The major difference between classical and molecular techniques is the greater diversity of genes that can be introduced by molecular techniques and the greater precision of these introductions. From a single gene to more than 50 genes can be introduced with the *Agrobacterium* system, although the site in the plant chromosome at which the foreign DNA has been integrated appears to be random. The donor DNA can be derived from the same or different plant species, or even from microorganisms or animal cells. For example, the DNA from fireflies (Ow et al., 1986) and bacteria (Konec et al., 1987) that codes for luminescence has been inserted into plants. Thus, no species barrier exists, because the chemical nature of DNA is inherent in its structure, irrespective of the organism of its origin. After being integrated, the gene, to be useful, must be expressed in the host plant. Genes have regions at one end of their nucleotide chain

that control when and under what conditions the gene will be expressed. These regions determine specific conditions for gene expression, for example, in the light, in specific tissues, or at certain stages of development (Goldberg et al., 1989). On the basis of this knowledge and recombinant DNA technology, one can attach the desired region of a gene to a bacterial gene and introduce the combination into a plant cell, where it will be expressed in a specific tissue. Particular conditions, such as wounding, may be needed for expression of the added gene or genes, and knowledge of these conditions can be used to precisely control expression. (Ryan, 1988).

GENOME MODIFICATION OF MICROORGANISMS—CLASSICAL TECHNIQUES

The classical methods of genome modification in microorganisms fall into two classes, selection of spontaneous and induced mutations and the exchange of DNA between (usually) closely related organisms. Spontaneous mutations result in a variety of heritable changes in the DNA, including the substitution of one nucleotide for another, the deletion or addition of one or more nucleotides, and other types of DNA rearrangements. Many spontaneous mutants appear to result from the movement of transposable elements to new locations in the cell's DNA. Transposable elements, first discovered in maize, also occur in other plants (McClintock, 1950), bacteria, and animals.

Another mechanism of generating variability in microorganisms is through the introduction of new genetic information from either chromosomal or plasmid DNA. DNA from a donor organism's chromosome is integrated into the recipient genome. Plasmids, being self-replicating, do not have to integrate their DNA into the genome of the recipient. Consequently, plasmid DNA can be transferred to more widely divergent organisms than DNA from the chromosome of a donor organism. Plasmid movement can be monitored because the DNA often provides the genetic code for readily distinguishable traits, such as antibiotic resistance.

In bacteria, gene transfer can occur by three different classical means: DNA-mediated transformation, in which the DNA is transferred as "naked" DNA; transduction, in which the DNA is enclosed in a virus coat and the virus mediates the transfer; and conjugation, in which the DNA is transferred during cell-to-cell contact between donor and recipient cells. Presumably, all these mechanisms operate in nature (Freifelder, 1987).

GENOME MODIFICATION OF MICROORGANISMS—MOLECULAR TECHNIQUES

The range of techniques to mutate bacteria has expanded and become sophisticated in recent years. It now is routine practice to mutate specific genes (insertion mutagenesis) (Ruvken and Ausubel, 1981) as well as to alter specific nucleotides within a gene (site-directed mutagenesis) (Kunkel, 1985). These techniques are possible not only for microbial genes, but, in principle, for genes from any organism.

The range of microorganisms among which DNA can be transferred has also been expanded through the use of new technologies. Thus, it is now possible to transform cells by physically altering their cell envelopes so that they become permeable to most DNA molecules. One such technique is electroporation, in which recipient cells and the genetic material to be transferred are subjected to an electric current (Fromm et al., 1987). The successful use of these techniques for genome modification requires that the entering DNA be able to replicate inside its new host. In principle, the techniques for performing these manipulations are straightforward. With such techniques, plasmids have been constructed that can replicate in both the bacterium *Escherichia coli* and the yeast *Saccharomyces cerevisiae* (Freifelder, 1987).

COMPARISON OF CLASSICAL AND MOLECULAR TECHNIQUES IN MICROORGANISMS

Recent molecular technological advances in mutagenesis and gene-transfer methods have opened new possibilities for expanding the range of microorganisms into which DNA from unrelated organisms can be introduced. The genus barrier and, indeed, the kingdom barrier are no longer complete obstacles.

Recombinant DNA methodology makes it possible to introduce pieces of DNA, consisting of either single or multiple genes, that can be defined in function and even in nucleotide sequence. With classical techniques of gene transfer, a variable number of genes can be transferred, the number depending on the mechanism of transfer; but predicting the precise number or the traits that have been transferred is difficult, and we cannot always predict the phenotypic expression that will result.

With classical methods of mutagenesis, chemical mutagens such as alkylating agents modify DNA in essentially random ways; it is not

possible to direct a mutation to specific genes, much less to specific sites within a gene. Indeed, one common alkylating agent alters a number of different genes simultaneously. These mutations can go unnoticed unless they produce phenotypic changes that make them detectable in their environments. Many mutations go undetected until the organisms are grown under conditions that support expression of the mutation.

SUMMARY

We have reviewed briefly the various means by which plants and microorganisms can be genetically modified by methods termed "classical" or "molecular." Genetic variability in microorganisms and plants is enhanced by classical modifications such as spontaneous or mutagen-induced variation, by hybridization, and by gene transfer. These methods are relatively imprecise and undirected and less powerful than molecular techniques for modifying genes. However, no conceptual distinction exists between genetic modification of plants and microorganisms by classical methods or by molecular techniques that modify DNA and transfer genes.

This understanding of the biological principles has the following implications for the report:

1. The deliberations of the committees were guided by the conclusion (NAS, 1987) that the *product* of genetic modification and selection should be the primary focus for making decisions about the environmental introduction of a plant or microorganism and not the *process* by which the products were obtained.

2. Information about the process used to produce a genetically modified organism is important in understanding the characteristics of the product. However, the nature of the process is not a useful criterion for determining whether the product requires less or more oversight.

3. The same physical and biological laws govern the response of organisms modified by modern molecular and cellular methods and those produced by classical methods, so exaggerated caution based only on speculation is unjustifiable. Scientists have vast experience with the products of classical modification, and the knowledge gained thereby is directly applicable to understanding, evaluation, and decision-making about the relative safety or risk of field tests on products of molecular modification techniques.

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