AGRICULTURAL BIOTECHNOLOGY SUPPORT PROJECT

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Introduction

This report outlines the activities and achievements of the Agricultural Biotechnology Support Project (ABSP) [DAN-A-00-00126-00 and 263-0240-G-00-6014-00] for the period from January through December 2001.

The primary goal of ABSP is:

To improve the capacity and policy environment for the use, management, and commercialization of agricultural biotechnology in developing countries and transition economies.

This goal is to be achieved by meeting the following two objectives:

1) *Establishment of a policy framework in developing countries and transition economies that promotes the use, management and commercialization of biotechnology by both host country and multinational agribusiness and research institutions.*

2) *Improvement of marketed crops through strategic research partnerships between the US and developing country public and private sectors.*

General Management Issues

ABSP Management Team Changes

Significant changes in the management team were faced by the ABSP in 2001. Dr. Johan Brink was offered and accepted the position of Co-Director of the Agricultural Biotechnology Support Project (ABSP). He joined ABSP from the Agriculture Research Council's Vegetable and Ornamental Plant Institute in South Africa where he was Assistant Director of Biotechnology. Dr. Brink took up his position at in the Institute of International Agriculture at MSU on June 1, 2001. Dr. Brink continued to take on more of the daily management duties of the project while the Director, Dr. Catherine Ives, focused primarily on documenting the success of the ABSP. At the end of June, Dr. Ives reduced her effort to the project to 50% for the period of July 1, 2001 onward, and due to personal commitments relocated to Boston at that time. She remained working closely with the ABSP team to ensure a smooth transition period, until leaving the project to take up a position as Boston as the Technology Licensing Associate for the Boston University Medical School in November 2001. Dr. Brink was appointed as ABSP Director with effect of December 1, 2001.

Dr. Andrea Johanson has continued to work at 0.75 FTE, responsible for reporting to USAID, the ABSP website, and giving technical oversight to the ASARECA program. Although planned, no additional support staff were hired in 2001. ABSP's Administrative Assistant, Ms. Sue Gibbons, continued with her responsibilities including bookkeeping and networking activities. Ms. Lacey continued to provide financial management to the project, managing the subcontracts. She will take the lead in closing out the various subcontracts in FY 2002/3.

Given new responsibilities in oversight on the ASARECA and SARB projects, the ABSP increased the level of effort of Dr. Pat Traynor, ABSP's Biosafety consultant, to 40% of her time, as well as provided additional clerical/logistical support to Virginia Tech. Dr. Traynor worked closely with Ms. Muffy Koch on the SARB project, as well as completing the biosafety manual (see project activities section for more details).

Dr. Joe Guenther of Idaho State University was hired, along with a graduate student at ISU, for a socioeconomic assessment on the deployment of transgenic potatoes in South Africa and Egypt. A final socioeconomic assessment was conducted on the deployment of multiple-virus resistant cucurbits in the Middle East.

AGERI, Egypt

During 2001 ABSP continued to coordinate with USAID/Cairo and its partner institution in Egypt, the Agricultural Genetic Engineering Research Institute (AGERI) to manage its stand-alone grant with the mission.

AGERI had proposed the purchase of a significant level of specialized equipment in 2000-2001; however, funding was not forthcoming so that process has been placed in

abeyance. The ABSP management and technology transfer staff were able to lend significant support to an intensive week-long specialized training program for 18 Egypt scientists and managers in the areas of Intellectual Property Management and Plant Variety Protection in September 2001. The events of September 11th required extensive changes in the program and travel plans for the two groups (see later). Dr. Maredia plans to travel to Egypt in Spring 2002 to follow-up on this program.

ABSP is working closely with AGERI and USAID/Cairo to rationally close-out the CUB program (scheduled to terminate September 30, 2002), recognizing that due to funding constraints all of the original objectives of the project will not have been met.

ABSP Database

In late 1997, it was determined that communication and office management would be better served with the creation of an ABSP database of contact information for electronic and postal communications, travel information, conference and workshop management and other information such as areas of expertise for ABSP contacts. The Management Team has continued to expand and develop the contacts and upgrade and improve the database management system. The database currently contains over 1115 contacts.

Subgrants

ABSP has continued to work closely with our subcontractors (VOPI, ASARECA, Monsanto, Virginia Tech, Cornell, TERI, and CABI) to execute, amend, and begin to close-out sub-agreements.

Reporting to USAID

The 2000 annual technical report was submitted to USAID in the first quarter of 2001. No Impact report was prepared this year. Instead detailed project documentation was prepared for USAID's External Evaluation of ABSP, which was held in June 2001.

USAID External Evaluation of ABSP

USAID requires routine technical and administrative management evaluations of its programs to examine the degree to which they are operating in accordance with agreement guidelines, and effectively carrying out the agenda set forth in the project proposal. In addition, end-of-project evaluations examine the impact of programs against USAID objectives and provide recommendations for future programs. The process of external review of ABSP to serve these three purposes began in 2000. Working with USAID, ABSP identified a team of potential candidates, developed a Scope of Work, and made the logistical arrangements for the evaluation.

Four esteemed specialists in their field reviewed the ABSP. The ABSP Evaluation Team consisted of:

Carliene Brenner

Consultant in Emerging Technologies Peniche L'Atelier , France

Ann Marie Thro
National Program Leader for Plant Genetics and Genomics USDA/CSREES

Ana Sittenfeld
President, Board of Directors Costa Rica -United States of America Foundation for Cooperation

Maria Jose Sampaio
EMBRAPA, Brazil and member of ABSP's External Board of Directors

The evaluation was held in two phases. In the first phase held June 11-13, 2001, the Evaluation Team met at Michigan State University for an overview of the project and individual presentations by US collaborators. Additionally, the team met with ABSP's Internal Board of Directors, the Acting Dean of the College of Agricultural and Natural Resources, and with M. Peter McPherson, President of MSU. The Evaluation Team examined project goals, objectives and work plans; the research program; assistance efforts in intellectual property and regulatory development; project management; training and institutional development; project impact; and financial management. The Evaluation Team then visited Dr. Molly Jahn at Cornell University from June 14-16 to review the work on the development of multi-virus resistant cucurbits.

In the second phase of the evaluation, held from July 8-14, the team split into two groups, with half of the team visiting Indonesia and half of the team visiting Egypt. The team then reunited in Washington, DC, to draft their final report and present preliminary findings to USAID and the ABSP Management Team.

The final report of the Evaluation Team was completed in September. The team congratulated USAID and MSU on successfully integrating research with policy issues.

The report highlighted the following additional successes:

- Stimulating awareness of biotechnology in developing countries;
- Successful training of developing country scientists;
- Supporting the development of regulatory procedures, institutions and infrastructure for biosafety and intellectual property rights (IPR) policy;
- Promoting public/private sector partnerships for research and technology development; and
- Supporting the development of technology transfer institutions in specific countries.

The team also pointed out weaknesses in the program, including a lack of emphasis on business development and the dilution of resources due to competing demands for resources and management time.

The Evaluation Team was impressed with the overall success of the ABSP and encouraged ABSP's Management Team to focus resources over the next year to document the experience of the ABSP in order to extend the benefits of the lessons

learned beyond its immediate partners. *A full copy of the Evaluation Report is available from ABSP or USAID on request.*

Annual External Board of Directors meetings

The annual meeting of ABSP's external Board of Directors was held on Thursday November 1, 2001 at USAID Washington D.C.

The current External Board is made up of the following individuals:

- **Mr. Mark Condon**
  Vice President, International Marketing
  American Seed Trade Association

- **Dr. Peter Matlon**
  Deputy Director Food Security
  Rockefeller Foundation

- **Dr. Donald Plucknett**
  Consultant, Agricultural Research & Development International

- **Dr. Michael Schechtman**
  Biotechnology & Scientific Services-USDA

- **Dr. Sally Van Wert**
  Trait Management, Aventis CropScience USA LP

- **Dr. Maria Jose Sampaio** *(not present at the November meeting)*
  EMBRAPA, Brazil

The meeting was attended by the External Board of Directors, ABSP management team members, and several ABSP collaborators, who gave presentations on various parts of ABSP's program:

Dan Clay, Director of MSU's Institute of International Agriculture opened the meeting and welcomed the participants.

Dr. Cherian George, Monsanto Company, and ***Dr. Vibha Dhawan***, from TERI, India spoke on the development of Vitamin A enhanced mustard oil for India, ABSP's newest research project.

Dr. Christopher Ngichabe, ASARECA Biotechnology Coordinator, presented information on the development of a biotechnology and biosafety program for Eastern and Central Africa.

Dr. Graham Thompson, ARC-Roodeplaat,VOPI, South Africa gave an update on the progress and plans of the Southern Africa Biosafety Program (SARB).

Dr. Magdy Madkour, Director of AGERI, addressed previous Board

recommendations and current program constraints.

Dr. Ann Marie Thro, a member of the External Evaluation Team, gave a presentation on the findings of the Team to the Board of Directors.

At the time of writing this report the External Board had yet to submit their recommendations from the meeting to USAID/ABSP.

A closed session was also held with the Board, USAID, MSU, and AGERI representatives to discuss the future of the ABSP/AGERI collaboration and the External Review of the ABSP.

Establishment of Policy Framework

Commercialization Study of AGERI, Egypt: Haas Business School

In May 1999 and May 2000, assessment teams from the International Business Development Program at the Haas School of Business at the University of California at Berkeley conducted assessments on the *Commercialization Prospects for AGERI* and *Development of a Strategic Marketing Plan*.

In 1999 the project team from the Haas School of Business conducted an analysis of the external and internal environment in which AGERI operates, primarily focusing on the organization's financial costs and revenue streams. The report indicated that AGERI could not be self-supporting if USAID/Cairo ceased support in 2001. From this report, USAID/Cairo initiated discussions and analysis on the establishment of a non-governmental organization (NGO), to be endowed by USAID.

In 2001 ABSP continued to work closely with the Haas Business School in identifying the team members and outlining the assignment for the final AGERI business management report. This report on the development of improved organizational structures and management strategies for AGERI, *AGERI: Strategic Marketing Plan,* represents the final assessment for AGERI, and concludes ABSP's relationship with UC-Berkeley's Business School. In developing the 2001 report the team engaged in both quantitative and qualitative research. Prior to the visit to Egypt the team conducted secondary research on AGERI, the various players in the agricultural biotechnology space, and general issues regarding genetic engineering of food crops. They also made initial contacts with agricultural industry specialists. They conducted 35 interviews with individuals in different sectors in Egypt: AGERI management and researchers; international and domestic agricultural biotechnology and seed industry organizations; and international, governmental and government-related agencies. The team also conducted some quantitative research using data from a wide variety of sources.

The team concluded that the value chain in which AGERI operates has three major components: the government, international agricultural biotechnology firms, and the domestic seed industry. AGERI is a small player near the top of this value chain, creating raw research and technology. In order for AGERI to be more market oriented, it

must work through the chain to gain greater access to end consumers and their needs as well as future market trends. In the report the team developed a model to mp market based criteria against product profitability to enable AGERI to evaluate its research projects. Applying this model to available information, their recommendation was for AGERI to focus on maize and fruits and vegetables. In addition, AGERI should focus domestically on input traits, i.e. those that benefit yield in relation to the agricultural constraints in Egypt.

The report made 7 over-arching recommendations for AGERI:

- Advance a market-driven philosophy by resolving the conflict between its governmental functions and self-sustainability.
- Expand management capability throughout the organization rather than focusing it near the top.
- Build strategic relationships across the value chain, especially through the local seed industry.
- Expand external communication efforts, both to shape public opinion of GM and also to learn about market needs.
- Evaluate projects based on a market-based metric.
- Promote international standards for intellectual property protection and biosafety regulation to encourage strong relationships with the private sector.
- Grow the Genetic Engineering Services Unit (GESU) as an additional source of revenue.

Dr. Catherine Ives traveled to Berkeley, CA for the presentation of this final report in October 2001. AGERI and USAID/Cairo are expected to use the three reports produced by the Haas Business School in formulating future support and instituting management changes.

Development of Biotechnology Initiative with ASARECA

ABSP has entered into a formal, contractual collaboration with the Association for Strengthening Agricultural Research in Eastern and Central Africa (ASARECA). As part of the process to develop a regional initiative in biotechnology and biosafety, ASARECA established a working group to examine issues and pragmatic approaches for integration of biotechnology through the existing regional networks and for the expansion of regional biosafety regulatory policy development.

Biotechnology Inventory

Also as part of the technical support process, ABSP developed An Inventory of Agricultural Biotechnology for the Eastern and Central Africa Region. The report highlights the current status of biotechnology applied to crops within ASARECA networks in order to give the working group an indication of the future potential of biotechnology tools for the improvement of crops that are important to Africa. In addition

to being available for download from the ABSP website (http://www.iia.msu.edu/absp/inventory1.html), the report was also published in 2001 as part of the USAID Africa Bureau's SD Publication Series (see later for reference).

This report has been widely praised by public and private sector organizations within Africa. AfricaBio, for example, has widely distributed the document to African public and private sector organizations.

ABSP Technical Support

In 2001 ABSP assisted in developing the terms of reference, for an half-time Coordinator for the ASARECA Biotechnology Working Group. ABSP also assisted in the screening and selection of applicants. Dr Christopher Ngichabe (KARI, Kenya) was selected and appointed to the position early in 2001.

In order to assist ASARECA, ABSP has provided technical support to the Biotechnology WG throughout the planning process. This has ensured that the WG members have been able to access international expertise in agricultural biotechnology and biosafety where necessary.

In 2001 ABSP assisted ASARECA in developing the terms of reference for two commissioned papers, one on biotechnology in the region, and one on biosafety. Dr. Andrea Johanson was selected by ASARECA to develop the paper that will assist the Working Group in identifying priority opportunities for research, adaptation of existing technology, and technology transfer. Ms Muffy Koch was selected to write the background paper on biosafety, examining the current status of biosafety in the region, outlining different options for the goals of a regional biosafety program and identifying strategic approaches to achieving each of the optional goals.

- **Regulatory Status And Regional Biosafety Regulatory Mechanism And Administration Under ASARECA**. *Muffy Koch, Innovation Biotechnologies, South Africa.*
- **Agricultural Biotechnology in the ASARECA Region: *Priorities for Research.*** *Andrea Johanson, ABSP, Michigan State University.*

The Biosafety paper was prepared and submitted to ASARECA at the end of 2001, and the Biotechnology paper is due to be submitted in January 2002. The papers will be used as discussion documents in working group meetings to be held in 2002. The outcome of the working group meetings will be a program for follow-up Stakeholder Workshops, one on biotechnology (to be held in June 2002), and one on biosafety (scheduled for August 2002).

The Southern Africa Regional Biosafety (SARB) Program

The purpose of this program is to: (1) build regional policy and technical capacity to support science-based regulation of the development, commercial application and trade in agricultural products derived from modern biotechnology; and (2) to lay the regulatory foundation that will support the field-testing of genetically engineered products in four of six target countries in the SADC region by 2003. By taking a regional approach, the program will provide a

foundation for later discussions of regulatory harmonization within SADC. SARB will promote science-based regulatory implementation and market access for biotechnology applications from both the public and private sectors. The program focuses on seven SADC countries: Zambia, Zimbabwe, Mozambique, Mauritius, Namibia, South Africa and Malawi.

The Vegetable and Ornamental Plant Institute (VOPI) of the Agricultural Research Council in South Africa is the lead contractor on the program. Innovation Biotechnology, a private consulting firm, is also involved in the development of the program.

Among Southern African countries, only Zimbabwe and South Africa currently have national biosafety regulations. Namibia, Mauritius, Zambia, and Tanzania are in various levels of regulatory development. It is notable that looking beyond legal regulatory development, however, only South Africa has extensive experience conducting regulatory reviews, field tests, and has taken products through to commercial approval. Several Southern African countries are currently examining approval of trials for biotechnology products including:

- Heartwater livestock vaccine (Zimbabwe, donor supported)
- Insect-resistant cotton (Zambia, private sector)
- Potatoes and sugar cane (Mauritius, public and private).

A total of 8 Southern African countries were visited during the period 28 January to 28 February 2001. In each country, meetings were held with officials of government departments such as Agriculture, Environment as well as with Biosafety regulators (if available) and members of Biosafety committees. The approach of countries with regard to Biosafety differs considerably and it is also evident that the level of co-ordination between government departments is not always effective. The dynamics of each country that were visited can now be much better appreciated. A series of workshops were held within SARB in 2001. Summaries of these are given below, and full details are provided in the technical section of this report.

SARB Regional Workshop

A highly successful workshop was held at the ARC Head Office, Pretoria, South Africa from 28 - 31 March 2001. The aim of this Workshop was to raise awareness for biosafety issues and to discuss the need and possibilities for a regional biosafety initiative. The Workshop was attended by 48 participants from: Botswana, Lesotho, Malawi, Mauritius, Mozambique, Namibia, South Africa, Swaziland, Tanzania, Zambia and Zimbabwe. Resource persons were present from Ghana, Lesotho, the Netherlands, South Africa, Uganda, the United States and Zimbabwe.

Regional Biosafety Risk Assessment Workshop

This course was held from 4 – 10 November 2001 at the ARC Head Office, Pretoria, South Africa. A total of 21 delegates from the seven core countries representing regulators and reviewers not previously trained, were invited but two failed to arrive. Additional representatives from the Department of Health in South Africa were also

invited. The course allowed for the first practical evaluation of the new ABSP Workbook for Biosafety Officials. The overall opinion was that the course was very successful and the Workbook very applicable. The numerous case studies that delegates had to review and give comments on resulted in strong interaction between them and the course presenters.

Journalists/ Media Workshop

The workshop was held at the ARC Head Office from 21 – 23 May 2001. A total of 17 journalists from 7 countries attended the workshop. This activity was combined with a USDA training course to be presented to scientists and policy makers on how to interact and communicate with the media. This workshop complimented the USDA course and allowed the media representatives from the core target countries to directly interact with scientists and policy makers.

Future activities in this regard will be coordinated with the AfricaBio/ Rockefeller Project.

National In-country Biosafety Training

The Work Group teams of each core country were tasked at the Work Group meeting in November 2000 to draw-up a work plan for their country regarding biosafety training. Most of these had been received by August 2001. The plans were reviewed by the SARB management team and recommendations made for revising the plans to each country. Once these had been accepted plans could then be made to implement them.

A schedule of in-country training has been developed. Dates are tentative and may change depending of availability of resource persons and outcomes of elections in Zambia and Zimbabwe. Namibia has requested that SARB support an application to conduct a field trial with a GMO as they have other sources of funding for biosafety training.

During 2001 two in-country training courses were successfully completed, one in Malawi, and one in Mozambique.

- An *In-Country Biosafety Capacity Building Workshop* was held in Lilongwe, Malawi, from 13 - 16 November 2001. The workshop dealt with the elementary introduction to biosafety and risk assessment to lay a foundation for them to begin developing their national strategy.
- An *In-Country Biosafety Capacity Building Workshop* was held in Maputo, Mozambique from 6 - 7 December 2001. There were 56 delegates from government, research facilities and seven of the 10 provinces. Consumers and environmental organizations were represented.

ABSP Biosafety Workbook

ABSP has continued working with Dr. Pat Traynor (ABSP's Biosafety expert) in the development of a "***Technical Workbook on Risk Assessment and Risk Management for Agricultural Biotechnology***." The workbook is designed to complement technical training for developing country scientists, Institutional Biosafety Committee (IBC)

members, and members of the National Biosafety Committees. It will provide supporting information for government biotechnology regulators and monitors. Additionally, it can serve as guidance for IBC members at U.S. academic and public sector institutions as well as reviewers on U.S. government agency biotechnology committees.

The main objectives of the workbook are:

- To provide a structured framework for an ABSP training program for biosafety review committees;
- To build the competence and confidence necessary for biosafety reviewers to conduct science-based reviews leading to appropriate decisions; and
- To develop materials that support ongoing training conducted by local organizations.

The team of authors, including Dr. Pat Traynor (Virginia Tech) Dr. Bob Fredericks (EPA), and Ms. Muffy Koch (Innovation Biotechnology, South Africa) has prepared a first draft of the workbook. The draft was circulated among the authors and revisions are currently being made. The edited draft was beta-tested at the Regional Biosafety Risk Assessment Workshop held by SARB in November 2001 at the ARC in South Africa (see above). Some changes and additions will be made to the workbook on the basis of feedback from course participants, and the new draft made available to reviewers early in 2002. It is hoped that the workbook will be completed and published by the fall of 2002.

IPR/Legal clearances for potatoes

During 2001, Dr Fred Erbisch completed an Intellectual Property 'audit' of MSU's potato tuber moth resistant B.t. potatoes. This is another step in the process of obtaining legal clearances to commercialize MSU's transgenic potatoes in Egypt, South Africa, and/or other developing countries. ABSP is continuing to work closely with Syngenta Company representatives to develop a road map for regulatory approval and IP clearance for commercialization of these B.t. potatoes in Egypt and South Africa.

Grades & Standards Assessments

In 2000/2001 ABSP sponsored four assessments on the need for and importance of grades and standards (G&S) for select commodities and countries in Africa. In 2000 ABSP sponsored two assessments on the need for and importance of grades and standards (G&S) for select commodities and countries in Africa. The first assessment was focused on fruits and vegetables in Kenya and the second assessment focused on a number of commodities in Malawi. In February 2001 similar assessments were carried out in Mozambique and Zambia. Summaries of the reports of the assessment teams follow. *Full reports of all G&S assessments are available on request--brief summaries follow:*

Zambia:

The Zambia study assesses whether more effective and efficient systems of Grades and Standards (G&S) can improve Zambia's agricultural sector and expand its trade and development opportunities. It identifies key actors, both public and private, and elaborates on their roles in developing G&S. An assessment of regional and international trends and trade issues for G&S provide the background for an outline of the important opportunities and constraints. The study briefly analyzes the structure, function, and flows of the supply chain for most of Zambia's agricultural products.

This analysis, by identifying critical leverage points that can catalyze change, makes recommendations for commodities where G&S initiatives can be most productively applied to achieve significant improvements in trade or social well being. Some of the most promising are presented in more detail as cost-benefit analyses. Finally, a series of crosscutting and cross-sectoral recommendations are offered for G&S improvements.

Overall the study concluded that G&S is a necessary but not sufficient factor to dramatically improve Zambia's competitive position. It must still address the formidable issues of inconsistent policy that does not foster investment and business development, non-performing public institutions, limited private sector capability and inadequate infrastructure. If Zambia undertakes to better understand and make use of G&S, it can utilize these strategic tools for product differentiation, market penetration, and system coordination, as well as quality and safety assurance. This applies to the domestic market as much as it does to its export markets

Mozambique:

The assessment team found that G&S are not generally perceived to be a high priority in Mozambique in the face of other constraints to production and marketing of agricultural commodities such as physical infrastructure, institutional infrastructure and low productivity.

The team found two additional factors that further exacerbate the overwhelming infrastructural impediments to a wide national-level appreciation of G&S issues are: (a) the lack of extensive forums about G&S issues at the national-level, and (b) the limited extent of collaboration between the different stakeholders involved in agricultural production and marketing in Mozambique.

In spite of the current lack of a significant national/regional momentum on G&S issues in Mozambique, the present research has revealed the strategic significance of G&S within specific subsectors in promoting agricultural/fishery export growth. The prawn, cotton and cashew subsectors are particular prominent in this regard as all three contribute significantly to the current export profile of Mozambique. A range of G&S issues impact significantly on these subsectors as they are predicted to with non-traditional commodities such as pigeon pea, sesame, banana, citrus, and paprika, which are all regarded as having export potential in the country.

N.B. The Grades and Standards component is now a part of the Partnerships for Food Industry Development program at Michigan State University (Director Lawrence Busch, email pfid@msu.edu).

Socioeconomic analyses

One of ABSP's goals is to improve marketed and food security crops in developing countries through the use of biotechnology and classical breeding. Dr. Molly Jahn, ABSP collaborator since 1996 and plant researcher at Cornell University, has developed multiple virus resistant cucurbit germplasm at the Cornell research facility. Dr. Jahn has also been very successful in building linkages with private seed companies, both in the U.S. and in several developing countries, for field-testing and commercialization of the resistant germplasm.

Virus diseases are a major limitation on worldwide production of cucurbits (cucumbers, melons and squashes). The ABSP supported socioeconomic assessments of virus-resistant cucurbits in Indonesia and South Africa in March and April 2001. The purpose of these assessments was to determine the potential benefit of virus disease resistant cultivars to farmers, consumers and seed companies in these two developing countries. Private seed companies in Indonesia and South Africa had already received multiple virus resistant cucurbit germplasm from Cornell prior to the socioeconomic assessment. This material was evaluated in the field for disease resistance and, concurrently, preliminary data was gathered to begin an assessment of the potential impact of the material on cucurbit yield, farmer income and consumers. The potential farm and market-level impacts will be used in an *ex ante* cost-benefit assessment of the investment by ABSP and seed companies in the development of these virus resistant cultivars. Given the highly speculative nature of predicting farmer adoption as well as farm-level impact of these cultivars, various scenarios (sets of key assumptions in the cost-benefit analysis) will be reported, along with their corresponding economic rates of return.

In each country the assessment was carried out by a multidisciplinary team, which included a socio-economist, a cucurbits breeder and the ABSP technology transfer coordinator. Professor Richard Bernsten, MSU Department of Agricultural Economics, traveled to Indonesia while David Mather, graduate student, MSU Dept. of Ag. Econ. and Karim Maredia, ABSP technology transfer coordinator, traveled to both countries. Mark Henning and George Moriarity, both researchers in Dr. Jahn's cucurbit breeding program at Cornell, traveled to Indonesia and South Africa, respectively. The full assessment team spent a week in each country, and the economists spent an additional week in country to collect statistical data.

The assessments in Indonesia was conducted in March 2001 in collaboration with the East-West Seed Company and the Ministry of Agriculture (AARD). The assessments in South Africa was conducted in April 2001 in collaboration with the Alpha Seed Company and the Agricultural Research Council (ARC).

The main objectives of the assessments were:

1. To investigate current status of cucurbits sub-sector in Indonesia and South Africa.
2. To evaluate and quantify potential/projected benefits and costs of the cucurbits technology to seed companies, producers, and consumers in Indonesia and

South Africa.

3. To identify key issues that ABSP researchers, the ABSP management team, and USAID need to take into account in the development and transfer of technology to developing countries in order to fully reap the potential benefits and minimize costs.

Ex Ante Assessment of the Impact of New Cucurbit Seed Varieties in Indonesia and South Africa

This study provided several key insights with respect to the characteristics of the cucurbit sub sector in Indonesia and South Africa, the performance of Cornell's disease-resistant germplasm in recent in-country field trials, the potential farm- and market-level economic impact of this germplasm, institutional lessons to date, and opportunities to increase the impact of Cornell collaboration with seed firms in these countries. Recent field tests of the Cornell materials demonstrate that their sources of resistance appear to be valuable in the Indonesian and South African growing environments. Under various cost-benefit analysis scenarios--each with strong assumptions (*i.e.*, minimal empirical data are available to estimate potential farm level benefits) regarding actual farm-level benefits as well as adoption rates--the rate of return to ABSP investment in the Cornell cucurbits program is positive.

With respect to poverty alleviation, the distribution of benefits and labor generation in Indonesia is ideal, as cucumber is produced by small farmers throughout the country, and consumed by all Indonesians. While melon is produced by small yet higher-resource farmers, it is consumed primarily by higher-income Indonesians. By contrast, the distribution of benefits in South Africa is less than ideal, as zucchini, cucumber and melon are produced by large, white farmers and consumed by high-income whites. While these crops do generate labor for black women, they are not traditionally grown by small farmers, and the constraints faced by these farmers to produce these high-value crops cannot be alleviated solely through varietal improvement. An exception to this is butternut, which is grown by both white and black farmers and consumed by all South Africans.

Keys to increasing future ABSP impact in cucurbits include: performing socioeconomic assessment of cucurbit subsectors in target countries to help set collaborative breeding priorities; encouraging Cornell to collaborate with multiple firms in each country, in order to avoid monopoly pricing of new technologies; and, in South Africa, to work with cucurbits that blacks traditionally produce and consume primarily pumpkins.

Economic Analysis Of Genetically Modified Potatoes in South Africa

Dr. Joseph F. Guenthner, and Ms Laura Louise Atchley from the Agricultural Economics at the University of Idaho carried out an economic analysis of the planned commercial release in South Africa in 2004 of the potato cultivars developed at MSU to be resistant to the potato tuber moth (*Phthoimaea operculella)*.

An ex-ante impact assessment was used to first evaluate the potential impact on the

individual farm level, followed by an analysis of the market level effects. The study then pursued the following objectives:

- To examine the current potato sub-sector in South Africa;
- To investigate and quantify potential benefits and costs of the technology to South African producers and consumers;
- To recognize central issues that the ASBP and researchers need to consider in the future development and technology transfer that will allow the greatest economic gain; and
- To use a market model to estimate the projected welfare effects to both producers and consumers.

The analysis estimated reductions of 9.9, 12.8, and 6.3 percent in variable costs to South African seed, commercial, and resource poor producers in the country. Expected discounted annual returns in Rand (1US$=10 Rand) are: seed producers would lose 970 thousand; the commercial producers would gain 10.8 million; resource poor producers would lose 2 million. Consumers would be expected to gain 15.8 million Rand annually. The total economic impact the ASBP project would be expected to have in South Africa during the twenty year period is 8.8 million US$. The gross annual benefit is 274 thousand US$ per year at a discount rate of 14.8% or 1.5 million US$ at a 6% discount rate. The estimated internal rates of return are 25% and 32% at the two discount rates, respectively.

This study will be presented in 2002 as a thesis for the Degree of Master of Agricultural Economics in the College of Graduate Studies, University of Idaho by Laura Louise Atchley.

Annual AUTM Meeting 2001

ABSP sponsored five participants to attend the annual the Association of University Technology Managers (AUTM) meeting in held in New Orleans, Louisiana USA from 2 February - 3 March 2001. The developing country participants included:

- *Dr. Achmand Fagi,* Agency for Agriculture Research and Development, Indonesia;
- *Dr. Tantono Subagyo*, KIAT Technology Transfer Office, Indonesia;
- *Dr. Gharib El-Karim*, Technology Transfer Office, AGERI, Egypt; and
- *Mr. Christo Kok*, IPR & Business Development Office, ARC -Roodeplaat, South Africa.

Dr. Karim Maredia, ABSP Technology Transfer Coordinator at MSU, also attended the Conference.

More than 1,500 participants attended the meeting that included plenary speakers, workshops, and a networking fair. The theme this year was "*AUTM Comes of Age*." In the past, AUTM, a nonprofit association with a membership of technology managers and

business executives who manage intellectual property, has focused primarily on what has been happening with IPR in the US economy. Currently, AUTM's fastest growing category of membership is from outside the USA. The AUTM Board of Director's has recognized the importance of this growing sector of members in the establishment and has recently created the position of Vice President for International Relations.

ABSP sponsored participants obtained a wealth of information from this year's meeting and spent a good deal of time networking with participants from all over the world. ABSP is working with various collaborators to exhibit technologies at next year's AUTM meeting.

Technology Transfer Workbook

ABSP's IP Consultant, Dr. Fred Erbisch, has taken the lead on the development of a training manual based on the experience gained during the six years of running the IP and Technology Transfer Course at MSU.

The proposed Intellectual Property Management Workbook will be a ready source for continued training and reference for participants on the MSU course and others like it. It will contain chapters on basic intellectual property and/or technology transfer management, each chapter beginning with a concise overview/definition of the topic, followed with a listing of key points or terms and include an explanation for each point or term. The next section will include a basic document or legal paper incorporating these points and terms. Case history examples and recommendations for avoiding problems will follow. The next to the last section will contain examples of good, poor and unacceptable points and terms which the reader will be asked to comment on, make suggestions for improvements, etc. An appendix to the workbook will contain answers and statements relating to these examples.

The last section of each chapter will consist of a number of questions relating directly to the reader's country's laws and customs as well as his/her organization's policies that will impact intellectual property management/technology transfer matters discussed in the chapter. The reader's answers can serve as a guide to his/her actual implementation of a particular practice in his/her country and/or organization.

The preliminary outline of the workbook is shown below:

1. Introduction – 4 major sections
   a. Objective of the workbook
   b. "How to use" the workbook
   c. What are intellectual properties
   d. What is technology transfer
2. Policy
3. Material Transfer Agreements
4. Confidential Disclosure Agreements
5. Research Agreements – covering only intellectual matters
6. Patents

7. Inventions
8. Plant Variety Protection
9. Copyrights and Trademarks
10. Licensing and License Agreements
11. Appendices – answers, explanations, comments on the assignments given at the end of each chapter.

It is anticipated that the Workbook will be between 80 and 100 pages in length. Draft chapters will be available for review beginning January 2002 and the completed Workbook will be available by the summer.

Bread for the World Institute Workshop

ABSP committed resources to assist the Bread for the World Institute (BFWI) in co-sponsoring a Conference on "Agricultural Biotechnology - Can it help reduce Hunger in Africa," to be held in held in Washington DC in March 2002. The conference is to be organized by BFWI through a grant from the Rockefeller Foundation and with support from ABSP. ABSP funding will cover the travel and expenses of 10 African delegates to attend the conference, which will explore the question "Can agricultural biotechnology can address hunger concerns in Africa?" Over 100 participants are expected to attend the meeting from local and international NGOs, universities, government and international agencies and institutions in Africa. ABSP has worked closely with the BFWI to develop the program for the meeting, to select speakers, and in the identification of the appropriate African participants to invite.

ABSP Case Studies

The ABSP Management Team have been working to develop several case study papers for publication in international development journals or some other appropriate forum to be identified. These papers will be case studies in the following areas:

- **Technology Transfer/IPR Activities** – the development of the technology transfer process and capacity building in IPR and technology transfer within ABSP.
- **Potato Case Study** – an overview of the development and deployment of the Bt potato tuber moth resistant potatoes developed at MSU.
- **Biosafety Case Study** – the development of biosafety frameworks and capacity building in biosafety in ABSP target countries.

CABI – *AgBioTechnet*

In 2001 ABSP discontinued support to CABI for *AgBiotechNet* and closed out this subaccount (see later in this report for a summary of activities.).

Food safety consultancy to Egypt

ABSP helped to recruit Dr. Hector Quemada, Crop Technology Inc., as a special consultant to assist the government of Egypt in the development of food safety guidelines and regulations for foods derived from GMOs. ABSP also assisted in the development of the scope of work for this assessment. USAID/Cairo supported this activity through the DAI/APRP policy project.

Research Collaboration Summaries

Please note that only research highlights are listed here. Full Technical Reports for all collaborations are appended to this Executive Report.

Amendments of subgrants to institutions involved in research collaborations with AGERI during this quarter were an important activity to promote the development of improved, marketed crops. As this is the final year of funding, it is important to develop appropriate close-out plans for the subagreements, and coordinate the technology transfer and IP agreements with the research agreements.

Under DAN-A-00-00126-00, research collaborations continued to focus on moving material from the research laboratories, greenhouses and fields in the US to developing countries. However, research will continue through June 2002 to update and improve material – both potatoes and cucurbits. In order to develop an orderly closeout of research projects, various sub-project Principle Investigators will be required to compose a final report. In addition, the ABSP Management will work closely with Principle Investigators and collaborating institutions to assure that intellectual property agreements are respected.

Potato Tuber Moth Resistant Potatoes

Michigan State University

Field trials conducted in 2001 at CIP-Egypt showed excellent control of PTM from MSU's transgenic lines. Storage experiments were also conducted with the harvested potatoes and the results of these tests were not yet available at the time of writing this report. A field trial was also planted in 2001 at ARC-Roodeplaat in South Africa (see below).

Seed production increases for year 2001 field trials in Egypt and South Africa were made at the MSU Potato Research Center, Montcalm Co., MI. Additionally, greenhouse tubers were produced by the private sector for field trials. Over 2000 seed pieces of various 'Spunta' lines (Spunta G2, Spunta G3, Spunta S1, Spunta S4 and Spunta 6a-3 [PVYcp/Cry5]) are now available, and plans are to test these lines in Egypt and South Africa for seed increase and test on commercial farms in South Africa in year August 2002. Planting these lines on commercial farms will allow growers to see the benefits of

these lines and hasten commercialization. Agronomic trials were conducted in Michigan testing the *Bt-c*ry5-Bt Spunta lines. All lines were comparable to the non-transformed control.

New constructs using four different promoters for *Bt-cry5* expression were developed. The different promoters include CaMV35s, Gelvin super promoter (GSP), Patatin, and Ubiquintin3. The Ubiquintin3 promoter was developed by USDA/ARS and thus eliminates several IPR constraints. This promoter is being used to design a freedom-to-operate vector, which may hasten commercialization of our transformed potato lines. Detached-leaf feeding bioassays are currently being carried out in the lab with 'Lady Rosetta' and 'Atlantic' lines transformed with the different constructs. The research team is also conducting experiments to use the MAT vector system, which would remove the selectable marker from the transgenic line during the transformation/regeneration steps.

New constructs using the same four promoters have also been developed for codon-modified *Bt-c*ry1Ac (J. Kemp, NMSU) expression, and transformation with these will begin in the near future.

Potato transformation with the different promoters and new genes are therefore showing excellent control of PTM. Seed increases of these lines will allow for test on grower's farms in Egypt and field tests in South Africa. This is an important step to commercialization as it allows the producer the opportunity to observe first hand the benefits of the product. Transforming the varieties 'Atlantic' and "Lady Rosetta" is also an important step towards commercialization in Egypt as these varieties are very important in the Egyptian chip industry. Mexico is also being considered for field-testing and commercialization, and some interest in this has been expressed by Frito-Lay, Inc.

South Africa

In South Africa during 2001 researchers continued the *in vitro* propagation of the potato material, and combined this with mini-tuber multiplication of the material in anticipation for planting in the fall. Field tests of the transgenic material were prepared and planted in September 2001. In order to assist South Africa in this effort, one of the VOPI researchers (Dr. Visser) visited MSU in July 2001 to observe field trials are conducted here.

Egypt

The MSU research team provided Bt. transgenic potato lines to test their resistance to PTM in field trials in Egypt after obtaining clearance from USAID, the Egyptian National Bio-safety Committee (NBC) and CIP-Lima. One trial was carried out at the AGERI Experimental plot and the other was conducted at the CIP station located in the middle of the Delta (potato growing area) for evaluation of the level of resistance to potato tuber moth (PTM). Field trials conducted in Feb. 2001 involved transgenic lines with different Bt -constructs and various gene promoters.

This year we focused on three lines derived from Spunta cultivar (SPG2, SPG3 and Sp-6A-3). The experiment at CIP site was conducted under natural infestation condition whereas in AGERI field plots were infested artificially. The insect damage on foliage was evaluated 3 times during the growing season. Trials were harvested on June 2001

and tubers were examined for PTM damage. Transformed potato tubers were harvested at AGERI and CIP-Egypt and results revealed that selected lines of Spunta have confirmed high level of resistance to PTM compared to control under field conditions.

Regeneration and transformation systems are now well established at AGERI for most of the important potato cultivars on the Egyptian market. Gene transfer is conducted through *Agrobacterium* -mediated transformation techniques.

The clearance of transgenic potato for food and feed safety and environmental assessment is still required by the Bio-safety Committee before taking steps towards the registration of Bt. transgenic lines for local commercial release. The Technology Transfer and Intellectual Property office at AGERI will help out with all the steps necessary for the commercialization of the transgenic product. The PTM transgenic potato lines can help to cut down the amount of seed potato imported annually from abroad (65000 ton).

AGERI will continue field-testing of PTM transgenic lines in 2002 to confirm the stability of genes and the level of resistance in collaboration with MSU potato team.

Virus Resistance in Cucurbits

Michigan State University

Research at MSU continued to study non-regeneration based transformation methods for cucurbits, but no stable transgenic plants were obtained using this method. However, *Agrobacterium* mediated transformation of cucumber has become increasingly efficient in the laboratory and at least seven different gene constructs have been transformed into monoecious (Straight 8) and gynoecious (GP14) genotypes. Mohamed Tawfik, an Egyptian Ph.D. student from AGERI has succeeded in introducing the *Arabidopsis CBF* (C-repeat binding factor) dehydration stress-related gene into cucumber. The CBF transcription factor is associated with increased freezing, drought, and salt stress by inducing expression of a number of genes that allow for the stress tolerance response. At this time, transfer of the introduced gene to the next generation has been verified for five T1 lines, and transgenic progeny from two T1 lines were subjected to salt stress treatments. The transgenic individuals showed significantly higher total reducing sugars (2-3 fold) and proline content (2-10 fold, depending on salt stress treatment) than did control, non-transgenic plants, and non-transgenic siblings from the transgenic families. These results indicate that presence of the *CBF* gene caused physiological adjustments associated with dehydration stress responses.

Cornell University

In 2001 Dr. Molly Jahn's research team at Cornell continued to run large greenhouse and field-based screens of advanced cucumber, melon and squash varieties with multiple resistance to virus and other diseases in order to identify the lines that are closest to commercial type and contain the broadest spectrum of disease resistance. The group hosted a major field day in Ithaca attended by 11 seed companies from around the world, including our ABSP cooperators from South Africa and Indonesia and Dr. Johan Brink and Dr. Karim Maredia of ABSP. Seed from this program has been sent

to many companies around the world that request it. At this time, over 1700 MTAs are listed for distribution of our breeding material on 6 continents. Simple one page material transfer agreements and two page commercial licenses have been developed and accepted by a broad range of companies in the developed and developing world. Trials conducted in cooperation with East West Seeds—Indonesia and Alpha Seeds in South Africa have gone well, and the research team have continued to work with each of these companies.

The team has also embarked on a new project to obtain multiple virus resistance in melons. A visiting scholar from Iran generated haploids from an F_1 population, a technique that has potential considerable potential for speeding the breeding process by generating homozygous virus resistant individuals.

AGERI, Egypt

In 2001 field trails were conducted at different locations, Giza (AGERI), Beni Suef (Sids), and Kalubia (El-Qanter) and verified that the transgenic squash line Eskandarani is highly tolerant to Zucchini Yellows Mosaic Virus (ZYMV-E) strain infection while maintaining high yield and superior marketable fruit quality. Therefore, this was followed by cross- pollination with those lines, which contain the *cp* gene of the most serious strain of ZYMV (ZYMV-E) for production of hybrid transgenic seeds for commercialization.

Greenhouse trails conducted for melon were completed and the obtained lines will be subjected to field evaluation as carried out for squash. In the case of watermelon, the introduction of the *cp* gene of ZYMV-E strain into the cv. Giza 1 of watermelon was successful. The transformed explants were successfully acclimatized followed by self-pollination of the Ro-transgenic lines.

Highlights:

- Production of hybrid squash lines tolerant to ZYMV-E strain by cross pollination between lines containing the *cp* gene of ZYMV-CT strain and others contain the *cp* gene of ZYMV-E strain.
- Evaluation of highly ZYMV-tolerant melon lines (R4-transgenic plants) containing the *cp* gene of ZYMV-CT strain under plastic greenhouse conditions.
- Introducing the *cp* gene of ZYMV-E strain into the cv. Giza 1 of watermelon followed by producing the Ro-transgenic line(s) and R1 seeds.
- Evaluation of virus resistance in the selected multiple virus squash, melon and cucumber lines obtained from Cornell University under greenhouse and field conditions.

Maize transformation for stem borer resistance

Work has continued at AGERI, Egypt during 2001 on the development of Egyptian maize lines with resistance to the corn *Sesamia cretica, Ostrinia nubilalis and Chilo agamnon*.

The main objectives have been to i) establish an efficient regeneration and transformation system(s) for Egyptian elite maize germplasm; ii) to introduce AGERI owned Bt gene(s) to confer resistance to stem borers; and iii) to evaluate the four novel constitutive maize promoters (outcome of Pioneer/AGERI collaboration) in driving the transient and stable expression of the GUS reporter gene in commercial maize hybrids.

Transformation experiments have been carried out using the biolistic gun for the reconfirmation of the transient GUS gene expression driven by the novel maize promoters in maize immature embryos and in tomato leaflets. Plasmid construction is now being continued for constructs that contain these novel promoters and GUS gene with the bar gene (with or without intron). The efficiency of these promoters in stable transformation of the bar gene will be tested.

The highly recalcitrant elite maize line Sd7 has been crossed with the American line A188, which is known to produce embryogenic calli. The regeneration frequency have been significantly improved it reached 62.33% in comparison to that of Sd7 (22.33%).

High Beta-Carotene Mustard Oil

ABSP has continued the research collaboration with Monsanto Company (St. Louis, MO) and the TATA Energy Research Institute (TERI, India) to develop high beta-carotene mustard. These mustard varieties will provide a food-based approach to reducing vitamin A deficiency in India. MSU finalized its contract with Monsanto and TERI in early 2001.

Mustard (*Brassica juncea*) is widely grown in India. There are several *B.juncea* mustard varieties grown in northern parts of India; among which three varieties, Varuna, Pusa Bold and RH30 are the most popular ones. These three genotypes (Varuna, Pusa Bold and RH30) were thus selected by TERI for transformation, who also supplied the necessary quantities of mustard seeds. Compared to canola (*Brassica napus*), the transformability of *Brassica juncea* is very poor. The genes coding for phytoene synthase (*Psy*) and phytoene desaturase (*CrtI*), both driven by a seed specific promoter, napin, were introduced into Indian mustard varieties to enhance the beta-carotene levels in their seeds. *Agrobacterium* transformation was used to transfer the genes into mustard.

Out of a total of over 100,000 explants from the three varieties that were infected during 2000-2001 period, fifty viable shoots were recovered. The fifty shoots yielded 8 orange seed-producing lines (seeds turn orange because of the higher carotenoid content), which included the three varieties (Varuna, Pusa Bold and RH30). About 100 T-2 generation plants from each of the eight orange seed producing T1 lines were screened for the absence of antibiotic marker gene.

The above technical work on transformation and screening of *Brassica juncea* was carried out at the Monsanto Research Center in Bangalore, India. Seeds from these plants are currently awaiting shipment to TERI for greenhouse and field trials. Meanwhile, TERI is engaged in securing necessary permits for transport of this transgenic mustard seeds to TERI research facilities.

Later in FY2002, ABSP and TERI will begin preliminary discussions to create awareness among government, industry, farmers groups and health NGOs about the development of this technology. A mid-term workshop is tentatively planned in FY2002 to discuss the benefits, issues, and concerns related to the nutritional modification of crops via agricultural biotechnology. ABSP, Monsanto and TERI will work together in 2002 to secure the appropriate regulatory approvals for the transgenic plants.

Developing Drought And Salinity Tolerant Wheat For Egyptian Agriculture

Research continued at AGERI, Egypt to enhance osmotic stress tolerance in Egyptian wheat crop through genetic transformation. This goal can be achieved by over expressing the key regulatory enzymes of the proline biosynthesis and sulfur assimilation pathways. The project aims to directly determine whether elevated levels of proline and active sulfur confer drought and salinity tolerance in wheat. Several genes conferring putative stress tolerance have been isolated and characterized by Prof. Desh Pal S. Verma's (Ohio State University) group for their role in conferring abiotic stress tolerance.

The team at AGERI has been successful in increasing regeneration and transformation efficiencies for Egyptian as well as American bread wheats by shortening the selection period, bombarding young immature embryos, using different regimes of hormones and allowing callus to recover for a week after bombardment. The Egyptian wheats (Giza 163 and Giza 164) have been transformed with salt-related genes (i.e., *mtlD* and *fructan-accumulating*, respectively) and promising results were obtained regarding tolerance against abiotic stresses.

Salinity experiments were conducted during winter 2001 to test the expression of stress tolerance genes in the two transgenics under stress. The overall results indicated the low performance of both transgenic plants under stress condition as indicated by the yield and its attributes, thus raising questions regarding the role of these genes in conferring tolerance against abiotic stress. However, more transgenics will be obtained and tested to reach a final decision whether to utilize these genes for further plant transformation experiments to improve tolerance against abiotic stresses in wheat as well as other cereals.

Tomato transformation for Development of Geminivirus Resistance

The research team at AGERI, Egypt have continued to make progress transgenic strategies to control tomato yellow leaf curl virus (TYLCV), a member of whitefly transmitted geminiviruses, one of the most devastating viral diseases of cultivated tomato, and vectored by the whitefly *Bemisia tabaci*.

One strategy investigated was the development of a recombinant DNA construct for resistance against TYLCV based on virus-induced expression of cytotoxin gene. Transformed plants resulting from cultivating T1 seeds in greenhouse biocontainment were challenged with viruliferous whiteflies together with a proper source of infection. Out of 83 transgenic plants, 62 showed no symptoms giving rise to a ratio of 3:1 as

expected in the T2. DNA was extracted from T2 transgenic tomato plants and evaluated using PCR/DNA probe to detect the introduced construct in the healthy plants. Seeds were collected from T2 transgenic tomato plants which showed resistance to whitefly transmitted geminivirus. The collected seeds were cultivated in a commercial greenhouse (as the local tomato cultivar, GH75, is a greenhouse cultivar) with source of infection. About 180 transgenic seeds from 25 lines as well as non-transgenic seeds (control) were cultivated in commercial greenhouse. Plants were subjected to natural infestation with the viruliferous whiteflies. After three months of transplantation, 57 plants out of 180 (31.6%) showed typical TYLCV symptoms. Seeds collected from healthy tomato plants will be used for crossing with the other parents after identifying the homologous plants for the introduced construct.

The virus-insect relationship has also been studied to find a way to control the insect vector of the virus. The acquisition access period required for transmission of TYLCV by *B. tabaci* was studied after feeding the insects on cotton plants for 45 day to obtain virus free whiteflies then fed on infected tomato plants with TYLCV to acquire the virus and insect samples were collected periodically. PCR was used to detect the viral genome after the different acquisition period using the viral coat protein specific primers. The expected 770 bp fragment was detected in DNA extracted from insects after 4 hrs feeding on infected tomato. It is well known that acquisition of a virus depends on the virus titer in the infected plant, the ability of insect to ingest the virus and the passage of the virus through the midgut wall and subsequent survival in the insect vector.

The ability of viruliferous whiteflies to inoculate tomato plants was also investigated, and fluorescence microscopy used to study the viral coat protein function in the viral circulation within the insect hemolymph.

Molecular Characterization of Insect Midgut Toxin Receptors

There are approximately 25 subspecies of *Bacillus thuringiensis* (Bt) that produce an array of parasporal crystalline proteins, known as Cry toxins, whish are lethal to a variety of agriculturally important insects. Transgenic plants carrying the toxin genes of Bt have now been released commercially in many countries around the world and efforts are underway to utilize such plants in Egypt and the Middle East for crop protection. Widespread use of Bt–based bioinsecticides may be causing insects to become resistant to certain Cry toxins. Designing new and novel Cry toxin molecules or synthetic substitutes derived from Cry toxins should help circumvent the potentially serious problem of insect resistance to Bt. The overall objective of the research projects at the University of Texas at Dallas in collaboration with AGERI, Egypt, is to determine the molecular mechanism(s) of insect resistance to the insecticidal toxins (Cry toxins) of *Bacillus thuringiensis*.

At AGERI selection for resistant cotton leaf worm by exposing larvae to Cry1C toxin for 25 generations has given rise to resistant strains showing an increased 10-fold tolerance to Bt toxin compared to the susceptible strain. The protein and DNA profiles of these resistant strains are currently being analyzed. Investigate the effects of metal ions and chelating agents on the integrity of BT-R_1 and Cry1Ab toxin binding to the molecule

Work at UT Dallas has continued to investigate the detailed biochemical processes that may be involved in resistance, in particular looking at the effect of calcium ions may influence the structure of binding sites of the BT toxin in the insect gut, and establishing models for the complex biochemical processes involved.

ABSP Networking & Communications

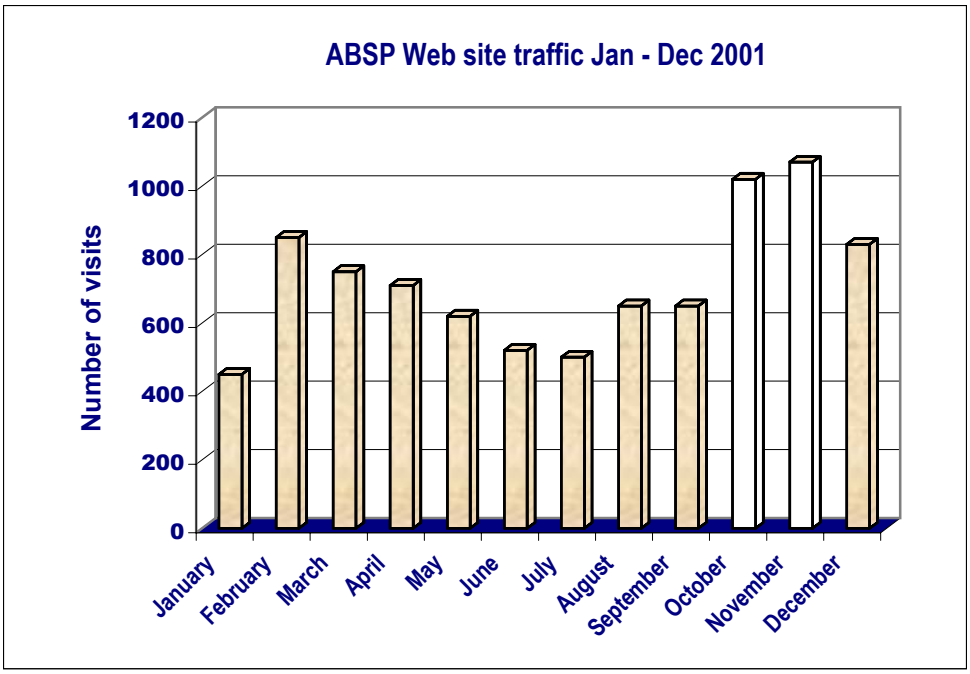
ABSP's World Wide Web pages

The ABSP Website continued to receive an increasing amount of traffic 2001. There have been over 17,000 visitors to the Website since August of 1999, with the average monthly number rising to a high of over 1,000 in November 2001. This coincides with a significant updating of the web pages, and the addition of a 'Links' page, giving links to other web sites on agricultural biotechnology and developing country issues. The most requested pages (not including the home page were the News (28%) pages where ABSP's newsletter *Linkages* is posted, followed by the Research pages (24%), Links (17%), Technology Transfer (11%), background (10%) and Policy Issues (9%).

Information on MSU's summer short courses in Intellectual Property Rights, Food Safety and Integrated Pest Management were also placed on the web site, with a new online registration enquiry form (http://www.iia.msu.edu/absp/msucourses02.html). This method of advertising the courses proved very effective, with over 60 applications to the courses being submitted directly from the website.

ABSP's *Agricultural Biotechnology Inventory for Africa*, made available on the web pages at the end of 2000 is still very popular and over 900 copies have been downloaded. Many of the major agricultural biotechnology websites now have a link to the ABSP site from their pages.

ABSP's website won a further award in 2001 – in February it was selected in Lightspan's StudyWeb® (http://studyweb.com/) as one of the best educational resources on the Web.



CABI's *AgbiotechNet*

In 2001 AgBiotechNet continued to deliver current information about biotechnology and biosafety for researchers, scientists and policy-makers worldwide, in both developed and developing countries. The site provides rapid and convenient access to research developments in genetic engineering and updates on economic and social issues as they affect developing and developed countries.

In 2001 AgBiotechNet had 447 subscriber registrations from institutions and individuals, over 1,600,759 successful hits on the site, approximately 2,800 per day. There have been 2984 direct *click-throughs* from AgBiotechNet to ABSP, since the site's launch, according to the hit counter on the *AgBiotechNet* site. User statistics have continued to show an extensive and increasing interest from users throughout the world.

AgBiotechNet has increased the information available on its site on biotech and developing countries. A hot topic on the subject, incorporating news, reviews, abstracts, and structured links is one of the most frequently visited pages on *AgBiotechNet*. This service is currently free, and highlights key issues relating to biotech and developing countries in an objective and informed way. It contains around 80 recent news stories, around 50 representative abstracts, links to around 20 reviews, around 100 key organizations, and 35 important external literature sources on agricultural biotechnology in developing countries. This is now automatically updated with the news, carrying around 10 stories a month, and has accumulated 15,562 visitor sessions in 2001. The page includes links to many key papers on developing country material.

Highlights from the Review Articles published in 2001 include:

- Public acceptance: a challenge for the strengthening of biotechnology R & D in

Brazil (Leila Macedo Oda and Bernardo E. C. Soares)

- Functional genomics for isolation of genes underlying QTL: disease resistance (David A Lightfoot)
- Biotechnology and sustainable agriculture (Antoinette M Mannion)
- Why US farmers have adopted genetically modified crops and the impact on US agriculture (Janet Carpenter and Leonard Gianessi)

User statistics from the year show that papers on developing country biotechnology were some of the most frequently downloaded. For example, the review "*Could agricultural biotechnology contribute to poverty alleviation?*" (Charles Spillane) has been downloaded 1,864 times in 2001.

In 2001 ABSP discontinued support to CABI for AgBiotechNet and closed out this subaccount.

LINKAGES Newsletter

The first electronic ABSP *LINKAGES* newsletter was distributed in April of 1999 to about 350 contacts. The newsletter and other special announcements, is sent quarterly by electronic mail and includes commentary from the ABSP Director, a feature article, and reports from ABSP domestic and international sources on current events and travel in the past quarter. ABSP now sends the electronic newsletter via a Yahoo Groups listserv. By using the listserv, it is now easier for people to subscribe and unsubscribe to the newsletter. *LINKAGES* is also posted to the ABSP web site under the ABSP News section (http://www.iia.msu.edu/absp/news.html). LINKAGES was distributed quarterly in 2001 to over 800 individuals and institutions worldwide. Feedback and responses from the newsletter have been very positive.

MSU Training Courses

Intellectual Property Rights (IPR) and Technology Transfer Internship Program (July 8 - 13, 2001)

The Institute of International Agriculture (IIA) and ABSP Project organized an Intellectual Property internship program from July 8 - 13, 2001 and was attended by 19 international participants from 10 countries including Costa Rica, Colombia, India, Kenya, Pakistan, Philippines, South Africa, Vietnam, Zimbabwe, and USA. Attached is a copy of the program along with the list of participants.

The internship IPR and TT internship program provided hand-on experience to international participants in day-to-day handling and management of intellectual properties in various settings. The ABSP sponsored participation of 4 participants from India and one from Colombia to the Intellectual Property Rights (IPR) and Technology Transfer Internship program.

Mr. Suresh Kumar
TIFAC, E-6 Qutab Hotel, New Delhi, India

Dr. Mercy Joseph
Center for Cellular and Molecular Biology, Hyderabad, India

Dr. Rajendra Jagdale
Director, Science and Technology Park, University of Pune, Maharashtra, India

Ms. Aarti Sharma
Scientific Affairs Specialist, Embassy of the United States of America, New Delhi, India

Ms. Maria Cardozo Mendoza
Advisor, Agrarian Development, Div. National Planning Dept., Colombia

Ms. Mendoza, an attorney in International Development in the Department of National Planning of Colombia in Bogotá, stated that she had attended courses before and understood the legal and technical language of IPR but this was the first time she had attended a course that had such practical case studies and applications to be practiced with other international participants from so many different countries.

Support to Technology Transfer Office, ARC Egypt
Dr. Fred Erbisch, MSU adjunct professor and ABSP consultant, spent 3 weeks in Egypt during the summer assisting the Agricultural Research Center (ARC) in developing basic materials and policy for its planned technology transfer office.

IPR and PVP Training, at MSU September 2001
As a result of the above consultation in Egypt for the ARC (Ministry of Agriculture), Drs. Erbisch and Karim Maredia, with ABSP staff support, developed and participated in two additional short-term training programs at Michigan State University in September 2001. One program was on intellectual property management and was presented to 9 representatives from the Egyptian ARC. The ARC will now establish a technology transfer office in Egypt that the September workshop participants will operate. The second training program dealt with plant variety protection (PVP) and was attended by 8 senior representatives from the ARC. Participants of this workshop will be staffing the Egyptian PVP Office.

ABSP participation in conferences & workshops

USAID Roundtable discussion on Biotechnology Strategies for Africa
On April 24, 2001, USAID hosted a roundtable on strategies for biotechnology in Africa, to initiate a dialog with U.S. and African constituencies on strategies for promoting access to agricultural biotechnology in Africa. There were 55 participants representing U.S. universities, PVOs, industry, other USG agencies, and African institutions. In addition, Senator Christopher Bond's staff delivered remarks, reiterating the strong Congressional interest in expanding agricultural biotechnology applications in developing countries.

The focus on Africa reflects a strong U.S. interest in the compelling potential benefit of biotechnology for Africa, and is a response to a specific Congressional directive to USAID to expand support for agricultural biotechnology. The goal of the roundtable was to develop a strategic framework that draws upon and builds on the Agency's experience in biotechnology, and more broadly in agriculture, to identify opportunities and approaches for future program development in Africa in partnership with various constituencies in the U.S. and in Africa.

Several of the speakers at the roundtable were from ABSP, or ABSP past and present collaborators. Speakers included the following:

- **Dr Catherine Ives** (MSU) -- ABSP: Linking biotech R&D to policy development.
- **Dr. Judith Chambers** (Monsanto) -- spoke on Public-Private Sector Collaboration: The example of Monsanto and KARI.
- **Dr. Andrea Johanson** (MSU) -- Opportunities for Technology Adaptation: ABSP Inventory of Biotech for Africa.
- **Dr. Geoffrey Mrema** (ASARECA) -- Strengthening Existing African Research Capacity: ASARECA experience.
- **Muffy Koch** (Innovation Biotechnology) -- Southern African Biosafety Training Program.

The dialog and discussions at the roundtable were in the following areas: USAID foundation of experience; integrated framework of interventions; linking national, regional, and global approaches; and principles for sustainable approaches. The roundtable concluded with consensus around various principles including the importance of building on existing African led efforts and an integrated approach that takes biotechnology from the laboratory to the farmer and public. The diverse expertise and constituency represented at the roundtable agreed on some key points and recommendations that should inform the strategic framework for USAID to integrate biotechnology into larger development goals in Africa. The highlights and recommendations are captured below.

Key recommendations to USAID from the meeting were:

- USAID needs to approach biotechnology development from a much broader context of goals and impacts, considering both potential benefits and risks against those goals.
- Biotechnology development must take an integrated approach, taking into account its specific components such as R&D, human and institutional capacity building, technology transfer, policy, regulatory issues, and the role of the private sector in technology delivery.
- It is clear that technology development drives policy formulation and is inextricably linked. The development of biosafety systems is, for example, both driven by and necessary for development of the technology.

- African leadership needs to be involved right from the start in biotechnology development; engaging in dialog and policy formulation with national, regional and international stakeholders. They must take the responsibility in decision and assessment of biotechnology in partnership with US and other interested parties.

Testimony before the U.S. House of Representatives

On September 25, 2001, Dr. Catherine Ives testified before the U.S. House of Representatives in a Congressional Committee hearing. The Science Committee's Subcommittee on Research held a hearing on two pieces of proposed legislation, H.R. 2051 (Strengthening National Science Foundation Sponsored Agricultural Biotechnology Research) and H.R. 2912 (Establishing a Grant Program for Partnerships between US Research Organizations and those in Developing Countries). Nick Smith (R-MI) and Bernice Johnson (D-TX), respectively, sponsored the bills.

The purpose of the hearing was to receive testimony regarding the legislation, which aims to expand the National Science Foundation's (NSF) investment in research related to plant genomics. H.R. 2051 authorized the NSF to establish regional plant genome and gene expression research and development centers to: (1) develop capabilities in basic plant genome research; (2) extend basic plant genomics research through plant breeding programs and accelerate its application to the development and testing of new varieties of enhanced food crops and crops that can be used as alternative energy sources; (3) develop alternative uses of agricultural crops; and (4) serve as centers for scientific and safety information on plant genomics.

H.R. 2912 authorized the NSF to establish research partnerships for supporting the development of plant research targeted to the needs of the developing world including: (1) basic genomic research on crops grown in the developing world; (2) development of plant biotechnologies that will advance and expedite the development of improved cultivars, including those that are pest-resistant, produce increased yield, or demonstrate increased tolerance to stress; (3) development of technologies to produce pharmaceutical compounds such as vaccines and medications in plants that can be grown in the developing world; and (4) research on the impact of plant biotechnology on the social, political, and economic conditions in countries in the developing world.

Also testifying were: Dr. Charles Arntzen (Arizona State University), Dr. Mary Clutter (NSF), and Dr. Robert Paarlberg (Wellesley College). The need to increased public investment in agricultural biotechnology was stressed and the ABSP was highlighted as a technology development and transfer program that is addressing the needs of developing countries.

Cassava Biotechnology Network Conference

Dr Andrea Johanson attended the Fifth Scientific Meeting of the Cassava Biotechnology Network (CBN) held at the Donald Danforth Plant Science Center in St. Louis, Missouri, USA, November 4-9, 2001. The conference was entitled *Cassava, An Ancient Crop for Modern Times: Food, Health, and Culture*. The first day of the conference was devoted to the presentation of cassava as a crop and constraints that limit productivity. The

succeeding four days were devoted to the current status of cassava biotechnology. The program will consist of keynote addresses, general lectures, specialized seminars, and poster sessions. A significant part of these sessions will be reserved for selected oral communications. In addition, there will be evening round tables to review progress and problems. All participants will receive the text of the communications at the beginning of the symposium.

AfricaBio Conference, South Africa

Dr. Johan Brink attended the AfricaBio *Biotechnology in Africa* Conference and present a paper on ABSP's Program in Africa titled: *ABSP: Building Capacity In Biotechnology And Biosafety In Africa*. The Conference was held in Johannesburg on the 26th and 27th of September 2001, and was attended by 192 delegates and about 15 media representatives of which over one hundred delegates represented 12 African countries. Present at this conference were renowned international speakers and over six South African speakers, covering different topics and issues. The ABSP presentation was very well received and stimulated numerous discussions. Nearly all of ABSP's partners in Africa attended this session and Dr John Wafula and Dr Christopher Ngichabe presented papers on ABSP's programs in Kenya and East Africa (ASARECA).

MSU Forum: Golden Rice—Will It Work?

Dr Andrea Johanson (ABSP) acted as Moderator for this forum, held on February 21, 2001 at the National Center for Food Safety and Toxicology (NCFST) at Michigan State University. The forum was sponsored by the NCFST and the Science, Religion and Society Committee of University Lutheran Church (ULC) and other campus ministries. In attendance were biological and social scientists, risk-assessment and food-law experts, clergy, and concerned citizens. Speakers at the forum included: Dr. Dean DellaPenna, Associate Professor of Biochemistry, Dr. Dale Harpstead, professor emeritus of crop and soil sciences; Dr. Pat Barnes McConnell, Professor of Resource Development; and Dr. Russell Freed, Professor of Crop and Soil Sciences.

MSU Crop and Soil Sciences/Horticulture Seminar

Dr Andrea Johanson (ABSP) gave a seminar on September 6, 2001 as part of the MSU Crop and Soil Science/Horticulture seminar series. The title of the presentation was "*Biotechnology for the Developing World: Is there More than Golden Rice*?"

ABSP Publications

Ives CL, A Johanson, J Lewis. 2001. Agricultural Biotechnology: A Review of Contemporary Issues. USAID, Bureau for Africa, Office of Sustainable Development, Agriculture, Natural Resources and Rural Enterprise Division, Washington D.C. pp. 29

Johanson, A and CL Ives. 2001. An Inventory of Agriculture Biotechnology for the Eastern and Central Africa Region. Technical paper No 114. USAID SD Publication Series, Office of Sustainable Development, Bureau of Africa, Washington D C.

Johanson, A. 2001. Agricultural Biotechnology in the ASARECA Region: Priorities for Research. *Discussion paper prepared for ASARECA Biotechnology Working Group*.

Lecoq H, Dafalla G, Desbiez C, Rip C, Delecolle B, Lanina T, Ullah Z, Grumet R. 2001. Biological and molecular characterization of Moroccan watermelon mosaic virus and a related Potyvirus isolate from Eastern Sudan. *Plant Disease* 85:547-552.

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Douches. DS, W Li, K Zarka, J Coombs, W Pett, E Grafius and T El-Nasr. 2001. Development of Bt-cry5 Insect Resistant Potato Lines Spunta-G2 and Spunta-G3. *HortSci. (in press)*.

El-Itriby HA, SK Assem, EHA Hussein, and MA Madkour 2001. The competence of maize shoot meristems for transformation of Egyptian maize inbred lines. *Arab Journal of Biotechnology*, 4(2): 149-162.

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Candas, M, BR Francis, NB Griko and LA Bulla, Jr. Calcium-dependent Cleavage of BT-R1, a Developmentally Cadherin in *Manduca sexta* Midgut Epithelium. J. Biol. Chem. (*Submitted)*

ABSP Travel Report 2001

Name	Institution	Country	Event	Destination
January 2001				
Catherine Ives	MSU	USA	USAID and AGERI meetings	Egypt
Walter Pett	Michigan State University	USA	Planting potatoes	Egypt
Francisco Santos	Michigan State University	USA	Plant potatoes	Egypt
February 2001				
Richard Bawden	Michigan State University	USA	Grades and standards	Mozambique
Gharib El-Karim	AGERI	EGYPT	AUTM	New Orleans
Mike Eustrom	USDA	USA	Grades & Standards	Zambia
Achmad Fagi	CRIFC	INDONESIA	AUTM	New Orleans
Daniele Giovannucci	World Bank	USA	Grades & Standards	Zambia
Hyde Haantuba	Ministry of Agriculture, Food	ZAMBIA	Grades & Standards	Zambia
Catherine Ives	MSU	USA	Haas School of Business	Berkley
Mylene Kherallah	IFPRI	USA	Grades and Standards	Mozambique
Christo Kok	ARC - Roodeplaat	SOUTH AFRICA	AUTM	New Orleans
Denis Mainville	Michigan State University	USA	Grades & Standards	Mozambique
Karim Maredia	Michigan State University	USA	IP Clearinghouse Meeting	Berkley
Karim Maredia	Michigan State University	USA	AUTM	New Orleans
Pat Sterns			Grades and Standards	Zambia
Tantono Subagyo	Agency for International Devt.	INDONESIA	AUTM	New Orleans
March 2001				
Richard Bernsten	Michigan State University	USA	Socioeconomic assessment	Indonesia
Johan Brink	Michigan State University	USA	ABSP Co-Director position meeting	East Lansing, MI
Mark Henning	Cornell University	USA	Socioeconomic assessment	Indonesia
Muhammad Herman	Research Institute for Food	INDONESIA	Socioeconomic assessment	Indonesia

Catherine Ives	MSU	USA	SARB Biosafety Workshop	Pretoria, South Africa
Molly Jahn	Cornell University	USA	Speaker at Hort. Seminar, MSU	East Lansing, MI
Karim Maredia	Michigan State University	USA	Socioeconomic assessment	Indonesia
David Mather	Michigan State University	USA	Socioeconomic assessment	Indonesia
April 2001				
W.S. Alhassan		GHANA	Biotech roundtable	Washington DC
Johan Brink	Michigan State University	SOUTH AFRICA	MSU Visit & Biotech Roundtable	East Lansing, MI
Catherine Ives	MSU	USA	Biotech Conference	Washington DC
Patricia Koch	Innovation Biotechnology	SOUTH AFRICA	Biotech Roundtable	Washington DC
Geoffrey Mrema	ASARECA	UGANDA	Biotech Roundtable	Washington DC
Christopher Ngichabe	ASARECA	KENYA	Biotech Roundtable	Washington DC
Theresa Sengooba	Namulonge Agricultural Research Inst.	UGANDA	Biotech Roundtable	Washington,DC
Patricia Traynor	VA Polytechnic Institute	USA	Speaker at Horticulture seminar	East Lansing, MI
Karim Maredia	MSU	USA	Visit to Cornell Cucurbit breeding program	Ithaca, NY
George Moriarty	Cornell	USA	Visit to Cornell Cucurbit breeding program	Ithaca. NY
May 2001				
Johan Brink	Michigan State University	USA	Start of new Co-Director of ABSP	East Lansing, MI
Catherine Ives	MSU	USA	Keystone Symposia/Plant Foods	Breckenridge
Francisco Santos	Michigan State University	USA	Research at AGERI and CIP	Giza, Egypt
June 2001				
John Barton	Stanford University	USA	ABSP External Evaluation	East Lansing, MI
Richard Bernsten	Michigan State University	USA	External Evaluation	Cornell Univ., NY
Johan Brink	Michigan State University	USA	Meet w/SARB	Pretoria, South Africa
Lee Bulla	University of Texas	USA	ABSP External Evaluation	East Lansing, MI
Melba Lacey	Michigan State University	USA	Bio 2001 International	San Diego CA
Magdy Madkour	ARC, Egypt	EGYPT	Bio 2001 International	San Diego CA

David Mather	Michigan State University	USA	External Evaluation	Cornell Univ., NY
Terry Meyer	Pioneer Hi-Bred	USA	ABSP External Evaluation	East Lansing, MI
Desh Pal Verma	Ohio State University	USA	ABSP External Evaluation	East Lansing, MI
Diedrich Visser	ARC - Roodeplaat	SOUTH AFRICA	1 week MSU potato lab & IPM	East Lansing, MI
July 2001				
Johan Brink	Michigan State University	USA	ABSP External Evaluation	Washington DC
Muhammad Herman	Research Institute for Food	INDONESIA	Annual meetings w/ABSP	East Lansing, MI
Catherine Ives	MSU	USA	ABSP External Evaluation	Washington DC
Catherine Ives	MSU	USA	ISNAR Biosafety workshop	Washington DC
Rajendra Jagdale	University of Pune	INDIA	MSU IPR Course	East Lansing, MI
Suresk Kumar	TIFAC	INDIA	MSU IPR Course	East Lansing, MI
Maria Mendoza	Department of National	COLOMBIA	MSU IPR Course	East Lansing, MI
Aarti Sharma	Embassy of the United	INDIA	MSU IPR Course	East Lansing, MI
Renette van Niekerk	ARC-Roodeplaat	SOUTH AFRICA	Training w/Melba on USAID	East Lansing, MI
August 2001				
Aernoudt Aardse	P.T. East West Seed	INDONESIA	Cornell Field Days	Ithaca, NY
Johan Brink	Michigan State University	USA	Cornell Field Days	Ithaca, NY
Bill Kerr	Alpha Seed Company	SOUTH AFRICA	Cornell Field Days	Ithaca, NY
Karim Maredia	Michigan State University	USA	Cornell Field Days	Ithaca, NY
September 2001				
Johan Brink	Michigan State University	USA	Africa Regional Biosafety< AfricaBio	Johannesburg, South Africa
Barry Greengrass	UPOV	FRANCE	IPR/PVP WORKSHOP	East Lansing, MI
Eid Megeed	Agriculture Research	EGYPT	IPR/PVP Workshop	East Lansing, MI
October 2001				
Johan Brink	Michigan State University	USA	ABSP Annual Board of Directors, CGIAR Centers Week	Washington DC
Vibha Dhawan	TERI	INDIA	External BOD Meeting	Washington DC
Susan Gibbons	Michigan State University	USA	USAID Budget Workshop	Arlington, VA

Catherine Ives	MSU	USA	Visit Haas School of Business	Berkeley, CA
Catherine Ives	MSU	USA	External BOD Meeting	Washington DC
Magdy Madkour	ARC	EGYPT	External BOD Meeting	Washington DC
Karim Maredia	Michigan State University	USA	CGIAR Centers Week	Washington DC
Christopher Ngichabe	ASARECA	KENYA	External BOD Meeting	Washington DC
Graham Thompson	ARC - Roodeplaat	SOUTH AFRICA	External BOD Meeting and MSU	Washington DC
November 2001				
Laura Atchley	University of Idaho	USA	Potato Assessment	Pretoria, South Africa
Johan Brink	Michigan State University	USA	Site visits & visit USAID	South Africa, Kenya, Uganda
Mark Condon	American Seed Trade	USA	External BOD Meeting	Washington DC
Cherian George	Monsanto	USA	External BOD Meeting	Washington DC
Joseph Guenthner	University of Idaho	USA	Potato Assessment	Pretoria. South Africa
Melba Lacey	Michigan State University	USA	External BOD Meeting	Washington DC
Karim Maredia	Michigan State University	USA	Potato Assessment	Pretoria, South Africa
Peter Matlon	Rockefeller Foundation	USA	External BOD Meeting	Washington DC
Michael Schechtman	Biotechnology & Scientific	USA	External BOD Meeting	Washington DC
Sally Van Wert	Aventis CropScience USA	USA	External BOD Meeting	Washington DC
December 2001				
John Kilama	Global BioDiversity Institute	USA	Site visits & visit USAID missions	Kenya, Uganda
Karim Maredia	Michigan State University	USA	Visit Teri Inst/Vit.A project	New Delhi, India

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Development of virus resistant cucurbit crops using a combination of molecular genetic and conventional breeding approaches

Principal Investigators:

Rebecca Grumet, Michigan State University

Molly Jahn, Cornell University

Project Partners:

Dr. Atek Sadik, AGERI, Egypt

Dr. Hamdy El-Doweny, Horticultural Research Inst., Egypt

Overall Project Goal:

Our goal is to develop virus resistant cucurbit crops using a combination of molecular genetic and conventional breeding approaches. We seek to develop novel transformation systems for cucurbit crops (Dr. R. Grumet, Michigan State University) and couple the transformation capacity with ongoing breeding efforts (Dr. M. Jahn, Cornell University) to develop high quality cucurbits with multiple virus and disease resistances.

Importance of the problem and rationale for approach:

Cucurbit species include a variety of high value crops (e.g., melons, watermelon, cucumber, summer squashes, winter squashes) that play important roles in both local diets and as export crops throughout the world. Currently a major limitation of successful production of these crops is infection by viruses including several potyviruses, including zucchini yellow mosaic virus (ZYMV) and the cucumovirus, cucumber mosaic virus. Crop losses of 50-100% in individual locations have been reported frequently.

During the past several years various groups, both commercial and public (including our group) have shown that it is possible to genetically engineer resistance to these viruses in cucurbit crops. In one case, virus resistant squash, originally released by the Asgrow Seed Company, has been produced commercially. A major limitation to more widespread use of this technology for various cucurbit crops, or improvement of cucurbit crops in general, is lack of efficient transformation systems. For several species there are no available transformation systems; for other species the transformation systems can be very inefficient and/or highly genotype specific. Often the difficulty in developing effective transformation systems lies in the tissue culture based process that requires successful regeneration from individual cells. In the past few years, new, non-regeneration dependent methods of plant transformation have been developed for a small number of species.

The primary motivating factors to develop such methods have been to bypass difficult and low efficiency regeneration protocols.

A current objective of this project is to establish efficient cucumber transformation system, with emphasis on the development of a novel, non-regeneration based system. Cucumber was chosen because of interest expressed by collaborating ABSP countries, Egypt and Indonesia. Two non-regeneration based approaches were tested: electrotransformation and pollen-tube mediated transformation. Electrotransformation was originally developed by Dr. Richard Allison (Michigan State University) for legume crops. Pollen tube transformation has been reported in China for several species including wheat, cotton, soybean rice and watermelon (Zeng et al. 1994; Huang et al. 1999; Hu and Wang 1999; Xie and Fan, 1992; Xu et al. 1991; Chen et al. 1999), but has received little attention in Western laboratories. If successful, the ability to avoid the time, effort, expense and sophistication necessary for regeneration-based systems, would make new transformation technology more readily transferrable to developing countries.

Previous research

In the first phase of the project, the biotechnology research focused on the development of zucchini yellow mosaic virus (ZYMV) resistant melons. Key activities included development of a regeneration and transformation system using melon leaf explants, production of transgenic melons via *Agrobacterium* mediated transformation of cotyledon or leaf explants, verification of transformation and gene expression, and virus screening of transgenic lines in greenhouse and field trials (Fang and Grumet, 1993; Grumet et al. 1995; Yadav et al. 1996). The melon transformation technology and the ZYMV coat protein construct were transferred to AGERI (Giza, Egypt) for use in melon and squash transformation. AGERI scientists have successfully produced transgenic squash lines showing resistance to ZYMV in the greenhouse and field.

In the current phase of the project our attention was focused primarily on the development of a non-regeneration dependent transformation system for cucumber as described above. Our initial goals were to determine the parameters appropriate for electrotransformation with cucumber including appropriate stage of seedling development, handling procedures to insure transformation and recovery, and the appropriate electrotransformation settings. Although analysis of vegetative tissue plants indicated a PCR-positive response for approximately 10% of the plants, we did not observe transfer of the gene to the next generation. The most likely explanation for the discrepancy between the vegetative tissue and the progeny was that the original treated plants were chimeric (i.e., containing a mixture of transformed and non-transformed tissue) and that the floral tissue had not been transformed. The electrotransformation system was then refined to utilize older seedlings at time when they are in the process of initiating floral primordia (7-10 days; Goffinet, 1990), and switching to a gynoecious breeding line which would allow for fruit set at the earliest nodes. We also tested different methods for pollen-tube mediated transformation including placing a droplet of solution with DNA on the surface of the style from which the stigma was excised following pollination, or injection of DNA into the ovary following pollination. Treatments were performed with circular and linearized plasmid DNA at 24 and 48 hours post-pollination using both methods.

Research Progress 2001

Non-regeneration based methods: Flowers were pollinated at the first four flowering nodes of the gynoecious electro-treated seedlings and fruit collected from approximately 200 plants. We did not obtain stable transgenic plants using this method.

Approximately 225 fruits were produced with different pollen tube treatments: droplet vs. injection, 24 vs. 48 h post-pollination, and circular vs. linear plasmid. Seedlings were screened for presence or expression of the introduced *Bar* gene by PCR, or exposing excised leaf punches to the herbicide Basta (glufosinate) and/or by direct spraying of seedlings in the growth chamber at the 1-2 leaf stage. Direct screening by PCR of seedlings from 27 fruit (30 seeds/fruit) did yield positive individuals. Leaf disc screening of 102 fruits (36 seeds/fruit) gave 12 fruits with one or more promising seedlings. Seedling screening of an additional 41 fruits (32 seeds/fruit) gave 12 fruits with one or more promising seedlings, all came from flowers treated with cut DNA. No differences were observed between 24 vs. 48 hours or injection vs. droplet. Putative positive individuals from the original leaf disk screen were grown for seed production and the second-generation progeny screened. Two sets of screening of these progeny did not give the expected 3/4 resistant individuals. Re-screening of families identified in the seedling assay also did not give reproducible results.

***Agrobacterium mediated transformation*:** *Agrobacterium*-mediated transformation of cucumber has become increasingly efficient in the laboratory and we have introduced at least seven different gene constructs into monoecious (Straight 8) and gynoecious (GP14) genotypes. Mohamed Tawfik, an Egyptian Ph.D. student from AGERI has introduced the *Arabidopsis CBF* (C-repeat binding factor) dehydration stress-related gene into cucumber. The CBF transcription factor is associated with increased freezing, drought, and salt stress by inducing expression of a number of genes that allow for the stress tolerance response (Thomashow, 1999). Successful transfer of the *CBF* gene has been verified by PCR analysis of 14 primary regenerants and expression verified for these individuals by northern analysis. T1 seed has been produced from 13 T0 individuals. At this time, transfer of the introduced gene to the next generation has been verified for five T1 lines; the ca. 3:1 ratios of PCR+/PCR- plants for these families are consistent with single gene integration.

Transgenic progeny from two T1 lines were subjected to salt stress treatments. The transgenic individuals showed significantly higher total reducing sugars (2-3 fold) and proline content (2-10 fold, depending on salt stress treatment) than did control, non-transgenic plants, and non-transgenic siblings from the transgenic families (Analysis of variance $P<0.001$). These results indicate that presence of the *CBF* gene caused physiological adjustments associated with dehydration stress responses.

Publications citing ABSP support during the reporting period:

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Papadopoulou E, Grumet R. 2001. Production of transgenic melon. In *Transgenic Plants and Crops*. Khachatouri GG, McHughen A, Scorza R, Nip YK, Hui YH (eds). Marcel Dekker Inc.

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Xu Y, Zhou C, Wang C, Li B. 1991. *Acta Hort Sinica* 18:49-54.

Yadav RC, Saleh MT, Grumet R. 1996. High frequency shoot regeneration from leaf explants of muskmelon. *Plant Cell Tis. Org. Cult.* 45:207-214.

Zheng JZ, Wang DJ, Wu YQ, Zhang J, Zhou WJ, Zhu XP, Xu NZ. 1994. Transgenic wheat plants obtained with pollen tube pathway method. *Science in China (Ser. B)* 37:319-325.

Breeding Cucurbits for Multiple Virus Resistance

Principle Investigator

Molly Jahn, Department of Plant Breeding, Cornell University

Key Personnel

George Moriarty

Mark Henning

Overview

In 2001 we continued to run large greenhouse and field-based screens of advanced cucumber, melon and squash varieties with multiple resistance to virus and other diseases in order to identify the lines that are closest to commercial type and contain the broadest spectrum of disease resistance. We hosted a major field day in Ithaca attended by 11 seed companies from around the world, including our ABSP cooperators from South Africa and Indonesia and Johan Brink and Karim Maredia. We have continued to send seed from this program to companies around the world that request it. At this time, we have over 1700 MTAs on our books for distribution of our breeding material on 6 continents. We have developed simple one page material transfer agreements and two page commercial licenses that have been accepted by a broad range of companies in the developed and developing world. The trials we conducted in cooperation with East West Seeds—Indonesia and Alpha Seeds in South Africa went well, and we have continued to work with each of these companies.

We also embarked on a new project to obtain multiple virus resistance in melons. We had a visiting scholar from Iran with us for 8 months who generated haploids from an F_1 population. This technique has potential for speeding the breeding process by generating homozygous virus resistant individuals.

Multi-Virus Resistant Topmark[1], Honeydew[2], Tam Uvalde[3], and Eastern Cantaloupes[4]

Topmark, Honeydew, and Tam Uvalde

In December 2000 we planted the best progenies we got from the 2000 field season in a virus

1 Topmark is an orange-fleshed melon with netting and no ribbing. It is a western shipper type, being suited for western growers and markets.
2 Honeydew is a green-fleshed melon with no netting or ribbing.
3 Tam Uvalde is a small, orange-fleshed melon, with heavy netting. Although not useful as an inbred, it combines very well to make nice hybrids.
4 Eastern cantaloupe is an orange-fleshed melon with netting and ribbing. It is suited for eastern growers and markets.

screen. This virus screen consisted of 10 Topmark, 24 honeydew, and 4 Tam Uvalde advanced progenies. In addition, we screened 4 F_2 Topmark progenies, which had been derived from resistant x resistant F_1s. We also screened 16 Honeydew F_2s, also derived from R x R F_1s. This material was screened individually in different combinations of the four viruses CMV, PRSV, WMV, and ZYMV. Depending on past virus tests, progenies were screened with anywhere from 1 to 4 of the viruses. Each progeny was always screened with the virus it had been screened with in the previous test.

In this past winter screen, resistant plants were selfed as well as intercrossed to boost levels of resistance. Only the most resistant plants selected from each virus screen were intercrossed within its background. This approach, which essentially combines backcross and pedigree methods, has worked well to boost levels of resistance in this program and also the squash virus and melon GSB programs.

From this winter test, we brought a number of progenies at varying generations in each background to the field in 2001:

Background	Generation	# of Progenies
Topmark	F_7	10
Topmark	F_1 (R x R)	3
Topmark	F_3	3
Honeydew	F_8, F_9	20
Honeydew	F_1 (R x R)	5
Honeydew	F_2	1
Honeydew	F_3	3
Tam Uvalde	F_7, F_{11}	3
Tam Uvalde	F_2	1

These progenies were inoculated individually with 1 to 4 viruses at the seedling stage before transplanting to the field the last week of June 2001. Each progeny was always screened with the virus it had been screened with in the previous test. For comparison, all plants from a progeny were transplanted regardless of whether they showed symptoms or not. In our field virus tests, there is a delay in the onset of symptoms, so it is not until the end of the season that we get an accurate picture of resistance. Also, fruit set will often cause onset of symptoms in previous symptomless plants, particularly with WMV.

In the Topmark background, virtually everything segregated for resistance to PRSV, WMV, and ZYMV. There were some progenies that were highly resistant to one or two viruses (80-90% of plants/progeny showing no symptoms). Some of the early generation material, which derives from

resistant x resistant crosses, looks promising for resistance. Much of this material has good resistance to powdery mildew. Unfortunately, on some of our better lines we did not get seed, so we had to go back one generation for our fall virus screen.

In the honeydew background, most progenies segregated for resistance. There were some advanced progenies that showed no symptoms to PRSV, WMV, and ZYMV. There were even some progenies that had high resistance to two viruses. Some of the early generation material, which derives from resistance x resistance crosses, looks promising for resistance to all four viruses.

In the Tam Uvalde background we have had less success in transferring resistance to the four viruses. This field season we had advanced progenies that segregated for WMV resistance and one progeny that showed no symptoms for ZYMV.

However, we have made up an excellent hybrid between a virus resistant Tam Uvalde and a netted, round, monecious, PMR Charentais type. The hybrid (01-907), akin to an eastern cantaloupe type, is ribbed, well netted, and slightly oblong with the fruits averaging almost 3 1/2 pounds. It has excellent quality, having a good combination of sweetness and flavor, with only a slight muskiness. It also has excellent depth of flesh. It had very vigorous vines that stood up till the end of the season and is highly resistant to powdery mildew. In addition, recent greenhouse screens clearly indicate resistance to ZYMV. There appears to be some resistance to PRSV and WMV. 907 is susceptible to CMV. For Fusarium, 907 is susceptible to race 1 but has some resistance to race 2.

01-907 F_1

In the above three backgrounds we recently completed the fall 2001 screen of plants from selections made in the field with very favorable results. It appears our strategy of intercrossing to combine and boost levels of virus resistance is working. We have very good levels of resistance for PRSV, WMV, and ZYMV in the Topmark and honeydew backgrounds. Most notably, in the Topmark background it appears we have combined resistance to PRSV, WMV, and ZYMV. In

honeydew, it seems we have combined PRV and WMV resistance. We also have some resistance to CMV in the Topmark and honeydew backgrounds. From our fall screen we have saved plants to self and intercross to boost levels of resistance.

Eastern Cantaloupes (Cornell 339 background)

In December 2000 we planted the best progenies we got from the 2000 field season in a virus screen. This consisted of 34 progenies representing various combinations of resistances at various generations. This material was screened individually in different combinations with the four viruses CMV, PRSV, WMV, and ZYMV. Resistant plants were selfed and intercrossed to boost levels of resistance. Only the most resistant plants selected from each virus screen were intercrossed within its background.

From this winter test, we brought a number of progenies at varying generations in the 339 background to the field in 2001:

Generations	# of Progenies
F_4, F_5, F_6, F_8	28
F_1 (R x R)	7
F_2	1

Virtually all 399 progenies segregated for virus resistance in the field. We recently completed the screen of plants from selections made in the field. We have good resistance to all four viruses, but the resistances are not yet combined in a homozygous state. We are currently selfing and intercrossing these progenies.

We have introduced 7 new recurrent parents to the multi-virus melon-breeding program. Three of these parents have Fusarium (0,2) resistance as well as PMR. These are the same parents being used in the GSB program. Last winter we made crosses to these parents using our most resistant material from the 2000 field season. The F_1s were selfed and we have recently screened a large F_2 population in the greenhouse. In this segregating population we have saved resistant individuals from each background and virus inoculation. We are backcrossing these to the appropriate recurrent parent.

Efficient Double Haploid Production in Melon Breeding for Multiple Virus Resistance

This was a collaborative project with Dr. Lisa Earle in the Dept. of Plant Breeding. Mahmoud Lotfi, a visiting scholar from the Dept. of Horticulture at University of Tehran, Iran (mlotfi@ut.ac.ir), conducted the research during his 8-month stay.

Use of doubled haploids to obtain inbred lines quickly has much promise in speeding the development of resistant varieties. However, the common technique of opening seeds to find haploid embryos (induced via irradiated pollen) is very time consuming and inefficient. The low rate

of induced embryos, difficulty in detecting and excising the embryos, and recovering enough plants has limited use of this procedure in breeding programs.

Two ways of producing doubled haploids— rescuing the embryos induced by irradiated pollen and then culturing them in liquid media under light, and ovule culture, were used to evaluate the real efficiency of using doubled haploids in melon breeding programs. The hope is to speed segregation and find desirable lines.

Our work shows that culture of seeds in liquid media three weeks after pollinating with irradiated pollen allows developing embryos to be easily detected after about 10 days. Embryos can be detected with light or by germination of the embryo. Embryos are not injured using this new method, and only those embryos that have good potential to develop are cultured. Using liquid culture is more efficient to obtain haploid embryos then opening seeds to find haploid embryos.

Two F_1 progenies from the melon virus project were used to obtain haploids using the liquid culture method. These F_1s were made from our resistant parents and 2 new recurrent parents. Using this new technique, we recovered (after 4 months) 155 haploid lines from 210 rescued embryos. We propagated these lines to 3-6 clones and then transplanted them to soil and then moved them to the greenhouse. The ovule culture proved to be mostly unsuccessful.

An attempt was made to double these haploids using a 0.5% colchicine emulsion, but unfortunately this was unsuccessful. We are currently trying lower levels of colchicine to induce doubling. We did have a small number of plants (about 10) that spontaneously doubled. Some have been checked with flow cytometry and they are true diploids. Once we successfully induce doubling we will self these plants and then screen the progeny for virus resistance in the greenhouse.

Multiple Disease Resistant Beit Alpha Cucumbers

We continue to try to combine target leafspot resistance with ZYMV resistance. Seminis will be running a TLS test for us in the next 3 months. We will be screening the same set of progenies for ZYMV resistance soon. In order to improve length and quality we also have crossed our multiple disease resistant Beit Alpha with a long Dutch greenhouse type.

International Agreements

We continue to send out numerous breeding lines for virus and disease resistance to a number of seed companies located outside the United States. Below is a list summarizing what we have sent:

Breeding Line(s)	Location
Multiple disease resistant North American slicing types	Denmark
C. pepo Eskandarany, *C. pepo*, multiple disease resistant Beit Alpha, pickle, and North American slicing cucumbers	Spain
Virus resistant melon, multiple disease	South Africa (2 cooperators)

resistant Beit Alpha, PMR C.pepo winter types	
Virus resistant melon, GSB resistant melon, *C. pepo* Eskandarany (for generation advance)	Brazil
C. pepo Eskandarany, make sterile *C. pepo* yellow, male sterile *C. pepo* caserta, *C. maxima*	Yugoslavia
PMR & ZYMV *C. pepo* yellow, PMR & ZYMV *C. pepo* caserta	Costa Rica (via U. of Wisconsin)

We also have had positive contact with Hytech Seeds in Egypt. They will be reviewing our 2001 Vegetable Breeding Report and plan to make seed requests. The difficulties we had previously encountered with respect to seed transfer appear to have been resolved as a consequence of a change in personnel at Hytech. We plan extensive cooperative trials for the 2002 season. This is an extremely positive development with respect to field-testing in Egypt

Multiple virus & disease resistance in *Cucurbita pepo* Eskanderany

Last winter selected material from this program was crossed to four commercial Eskanderany F_1s to improve type. Sixty-four lines resulting from these crosses were planted in the field without virus pressure and selfed. Selections were made based on plant habit, mildew resistance, and fruit characteristics resulting in 82 F_2 families which are being tested with all 4 viruses separately this winter. Resistant selections will be retested and planted to our 2002 field trial.

Also 45 selections from the 2000 Eskanderany program were tested for virus resistance in the winter greenhouse screen. Resistant selections were selfed and intercrossed resulting in 77 breeding lines in the BC_2 F_2 through BC_3 F_4 generation that were tested in the summer of 2001. Resistant selections from the summer program will be replanted in the spring of 2002 and incorporated with the resistant selections from the winter virus screens and should result in better fruit and plant characteristics.

Multiple virus & disease resistance in *Cucurbita moschata*

South African tests of our breeding material showed them to be lacking in resistance to WMV Moroccan strain. Our source of virus resistance for all four major cucurbit viruses, ***C. moschata* Nigerian Local** was sent to the Vegetable & Ornamental Plant Institute in Pretoria, South Africa to be tested for this isolate. Nigerian Local was found to be resistant to WMV II Moroccan strain and has been incorporated into the breeding programs of our South African cooperators. Requests have been received for Butternut material derived from crosses with Nigerian Local for trials in Johannesburg and KwaZulu-Natal.

Development of virus resistant cucurbit crops using a combination of molecular genetics and conventional breeding approaches

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Overall project goal

The main goal is development of virus resistance in some cucurbit plants *via* genetic engineering approaches by introducing the coat protein gene of ZYMV-CT strain into squash plants cv. Eskandarani, melon cv. Shahd EL-Dokki and/or Annas, cucumber cv. Beit Alpha MR and watermelon cvs. Giza 1 and Giza 21.

Importance of the problem

In Egypt, the cultivated cucurbits are infected with some common viruses, i.e., zucchini yellow mosaic potyvirus (ZYMV), cucumber mosaic cucumovirus (CMV) and/or watermelon mosaic potyvirus (WMV) in common, causing reduction in yield and quality. The control of such viruses based on using insecticides and/or inspection and roguing was ineffective. Therefore, the

developed of cultivars resistant to such viruses would be the most effective means for its control.

Project background

Control of viral diseases through using the classical methods was not effective enough (Sherf and MacNab, 1986), but some limited success has been obtained with the use of mineral oil sprays (Webb and Linda, 1993). Therefore, introducing the viral coat protein (Clough and Hamm, 1995) *via* genetic engineering approaches followed by developing resistant transgenic plants would be the possible future option for control of plant RNA viruses. Coat protein-mediated protection (CPMP) (Gonsalves and Slightom, 1993; Fitch and Beachy, 1993 and Lomonoscoff, 1995) constitutes a.major breakthrough to facilitate the incorporation of virus resistance in crop plants.

Provvidenti *et al*. (1984) reported that ZYMV was the most serious virus affecting squash, muskmelon (*Cucumis melon* L.) and other cucurbits. Khalil *et al*. (1999) produced transgenic squash plants resistant to ZYMV-CT strain under the ABSP/AGERI project.

Rational for approach

In Egypt, the area under cultivation with squash crop is around 78,000 feddans producing about 568,000 tons. In addition, the export values for melon and watermelon exceed $1 million annually ($ 749,000 and $ 310,000, respectively). These crops can be completely destroyed when infected by ZYMV, CMV and WMV. Transgenic varieties that are virus resistant, based on the incorporation of virus-derived transgenes has been widely demonstrated to be an effective strategy for control of such viruses and could increase productivity and reduce inputs.

Previous research

1. Successful introduction of ZYMV-CT-*cp* gene, which was kindly provided by Dr. R. Grumet, Dept. Horticulture and Vegetable Gene. Lab., Michigan State Univ., East Lansing, USA into the Egyptian Eskandarani cultivar of squash and Shahd-el-Dokki cultivar of melon *via Agrobacterium*-mediated gene transfer system using shoot tip explants and *A. tumefaciens* LBA4404 strain harboring the pGA643 plasmid. The impact of this research was:
   a) Production of transgenic squash lines highly tolerant to ZYMV-E strain.
   b) Evaluation of the resistance to ZYMV-E strain was performed under greenhouse for R1, R2, R3 and R4 plants at AGERI followed by field trials at Sids Experimental Station for three seasons (R5, R6 and R7).
   c) Evaluation of the resistance to ZYMV-E strain of R5 squash plants under small-scale contained open field conditions at different locations (El-Qanater and El-Nobaria).
   d) The selected lines gave good marketable fruits with a high yield. It is important to mention that the non-transgenic plants were destroyed after 2-3 weeks from ZYMV infection and no yield was obtained.

2. Establishment of regeneration system in cucumber.
3. Establishment of transformation system in cucumber.
4. Evaluation of Ro-transformed cucumber plants.
5. Establishment of regeneration system in watermelon.
6. Establishment of transformation system in watermelon.
7. Isolation, identification and purification of watermelon mosaic potyvirus (WMV)
8. Design and synthesis of primers specific to the *cp* gene of WMV for RT-PCR.
9. Evaluation of the multiple virus resistant squash, melon and cucumber lines obtained from Cornell University under field conditions.

Specific project objectives

- Re-evaluation of the highly ZYMV-tolerant squash lines of R7 in the summer season (April-July, 2001) at different locations.
- Production of hybrid squash lines tolerant to ZYMV-E strain.
- Evaluation of highly ZYMV-tolerant melon lines containing the *cp* gene of ZYMV-CT strain (R4) under plastic greenhouse conditions.
- Introducing the *cp* gene of ZYMV into a watermelon cultivar.
- Evaluation of the multiple resistant squash, melon and cucumber lines obtained from Cornell University under greenhouse and/or field conditions.

Research progress

Objective 1: Re-evaluation of highly ZYMV-tolerant squash lines of R7 in the summer season (April-July, 2001) at different locations

In the period from April to July 2001, two sets of R7-squash seeds belonging to the highly ZYMV-tolerant squash line 5 and containing the *cp* gene of ZYMV-CT strain were planted and evaluated for virus resistance under field conditions at different locations, i.e., Sids Experimental Station, Beni Suef (Figure 1); AGERI, Giza and El-Qanater Experimental Station, El-Kalubia (Figure 2) in the presence of non-transgenic plants as control. Results showed that the majority of the tested plants showed high resistance to ZYMV-E strain as no symptoms 12 weeks post planting were observed. It was also noted that the plants appeared healthy at Beni Suef and El-Qanater, respectively, compared with those planted at AGERI (Giza). This could be due to the high percentage of salts in AGERI's soil. The ratio of virus-infected plants after 10 weeks from planting was 2, 8 and 10% at the three different locations, respectively. A large number of squash plants showed no symptoms after 14 weeks from planting. Marketable squash fruits as well as good yield judged based on the horticultural characters of the control plants were obtained as represented in Figures 1 and 2. At the same time R5-squash seeds belonging to the highly ZYMV-tolerant squash belonging to lines 8 and 9, and containing the *cp* gene of ZYMV-E strain (unpublished data) were also planted to serve in the production of hybrid lines in September 2001.

A

B

Figure 1. A) Evaluation of R7-transgenic squash plants of the highly ZYMV-tolerant line under field conditions at Sids Experimental Station, Egypt in the summer season (April-July, 2001). **B)** Marketable transgenic squash fruits of R7-transgenic squash plants.

Figure 2. A) Evaluation of R7-transgenic squash plants of the highly ZYMV-tolerant line under field conditions at El-Qanater Experimental Station, Egypt in the summer season (April-July, 2001). B) Marketable transgenic squash fruits of R7-transgenic squash plants.

Objective 2: Production of hybrid squash lines tolerant to ZYMV-E strain

For such purpose, Prof. Dr. El-Downey, HRI, preferred to produce the hybrid lines by cross pollination between the highly ZYMV-tolerant squash lines of R7, which contain the ZYMV-CT-*cp* gene (Lines 1 and 4) and those belonging to R5 plants, which contain the ZYMV-E-*cp* gene (Lines 8 and 9, unpublished data). Data in Figure 3 show squash lines, with virus resistance up to 14 week from planting. These lines were selected for producing hybrid seeds under open field conditions at AGERI and Sids Experimental Station (Figure 4) in September 2001.

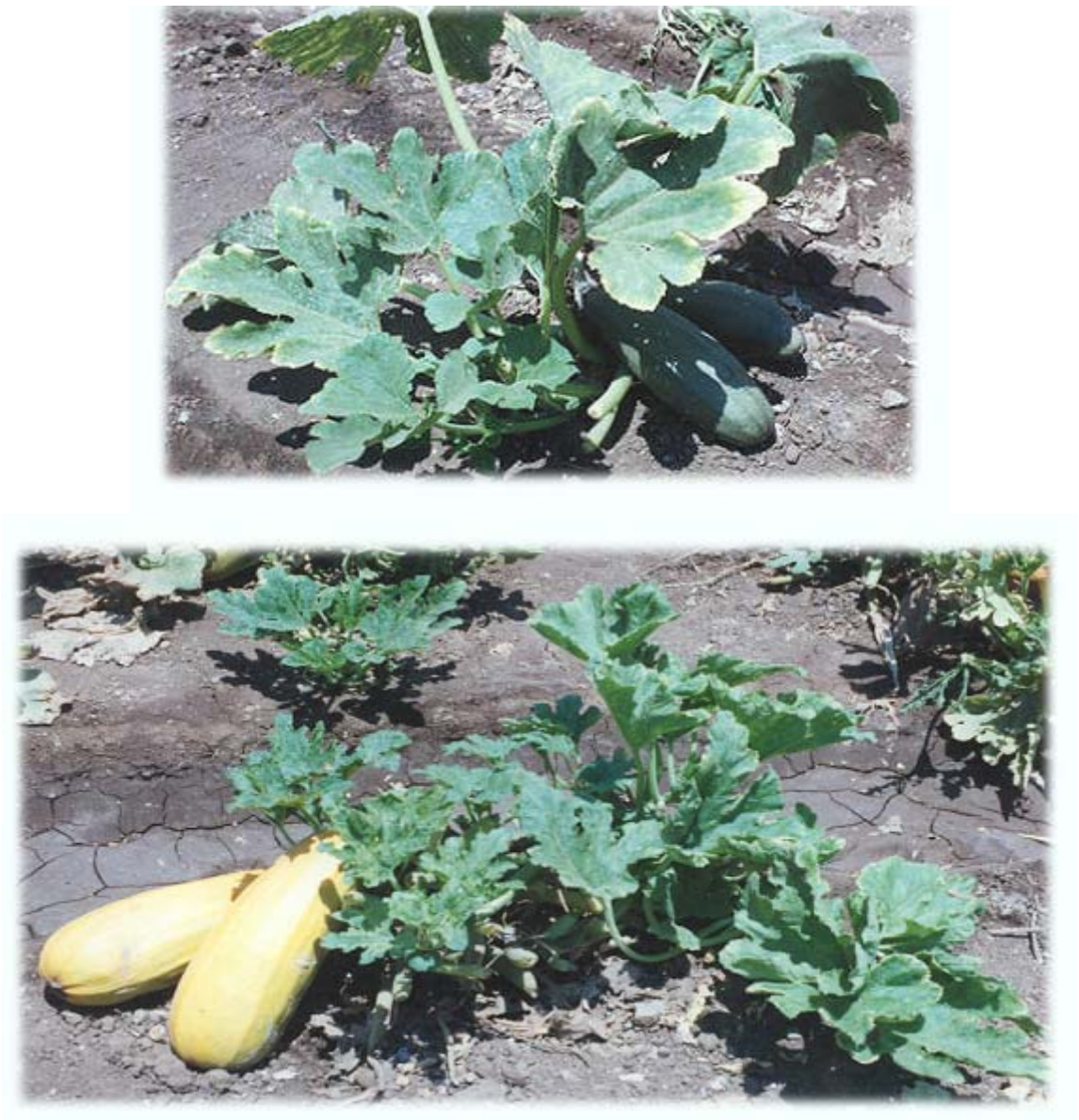

Figure 3. Seed production of highly ZYMV-tolerant squash plants during the summer season at AGERI under the open field conditions. Upper, fruits contain the ZYMV-*cp* gene of the Egyptian

strain. Lower, fruits contain the ZYMV-CT-*cp* gene belonging to R7-transgenic squash plants. Note: these lines were selected based on their high resistance to ZYMV-E strain for production of hybrid lines in September 2001.

Figure 4: Production of hybrid squash lines at Sids Experimental Station, Beni Suef, Egypt.

Objective 3: Evaluation of highly ZYMV-tolerant melon lines containing the *cp* gene of ZYMV-CT strain (R4) under plastic greenhouse conditions

In this experiment, 18-21 plants belonging to R4-transgenic lines of melon (cv. Shad El-Dokki) named M2000 and M1 (lines M3, M4 M11 and M12, respectively) were evaluated for virus resistance in three replicates under greenhouse conditions at AGERI and El-Dokki Experimental Station. It is worth monotonies that these lines were left without any virus inoculation treatment and ZYMV-infected non-transgenic melon plants were also used as a source of virus infection. Results in Figure 5 shows that most of the evaluated plants showed high virus resistance as no symptoms were observed until the 10th week of planting. Fruits with good horticultural characteristics were obtained as shown in Figure 6. It was observed that some plants were sensitive to the soil salts.

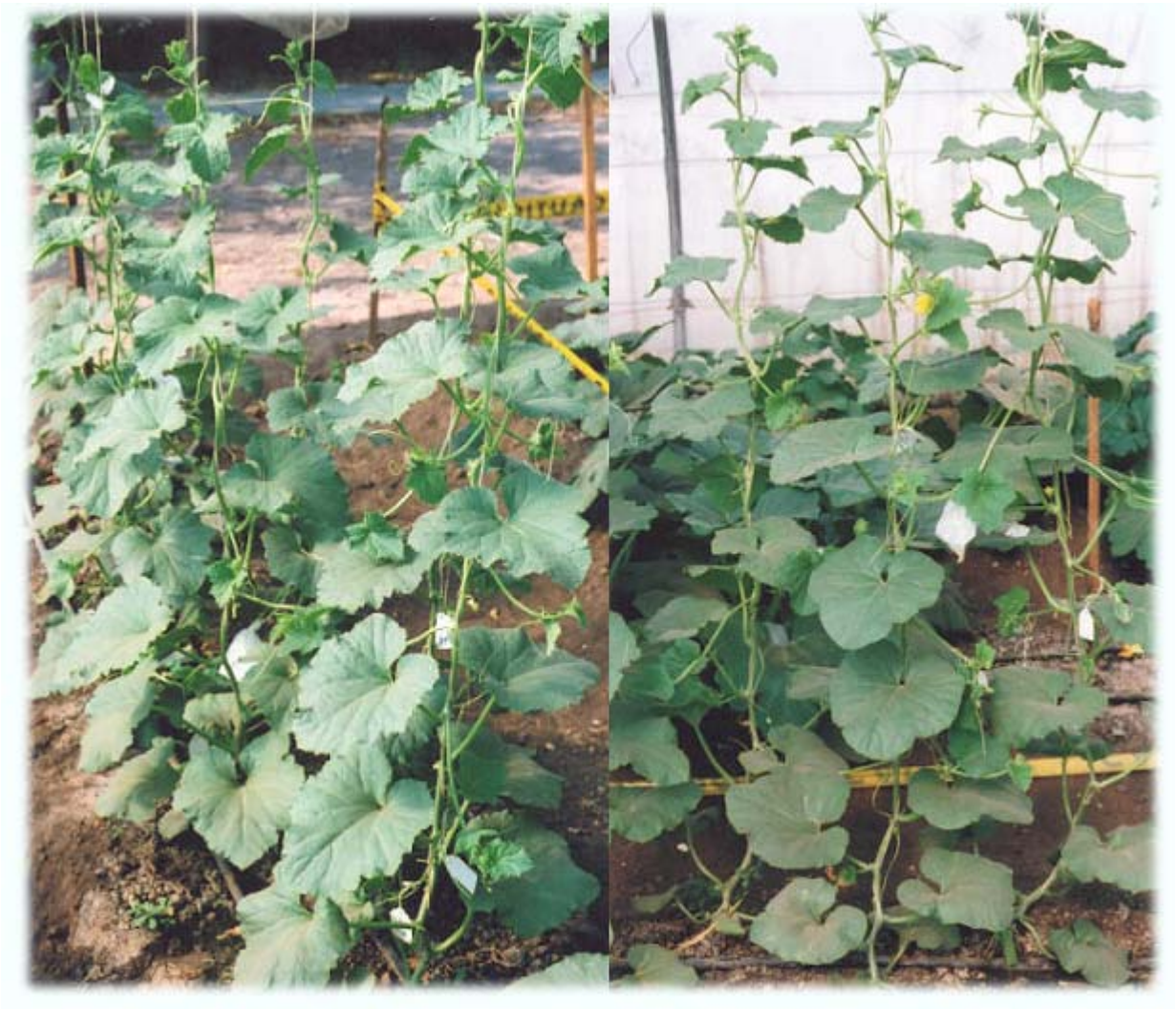

Figure 5. Evaluation of virus resistance of R4-transgenic melon plants to ZYMV-E strain in the summer season, 2001 at AGERI, Egypt under plastic greenhouse.

Figure 6. Fruits and seeds of R4-transgenic melon plants highly tolerant to ZYMV-E strain, and contain the ZYMV-CT-*cp* gene.

Objective 4: Introducing the *cp* gene of ZYMV into a watermelon cultivar

The ZYMV-E-*cp* gene was introduced into Giza1 watermelon cultivar. Data in Figures 7 and 8 show the steps of acclimatization followed by fruit and seeds production. We are going to introduce the ZYMV-CT-*cp* gene *via Agrobacterium*-mediated gene transfer system, in order to produce a hybrid watermelon cultivar containing the *cp* genes of CT and E chains of ZYMV.

Figure 7: Acclimatization of Ro-transgenic watermelon lines of the cv. Giza 1 containing the *cp* gene of ZYMV-E strain grown under biocontainment conditions.

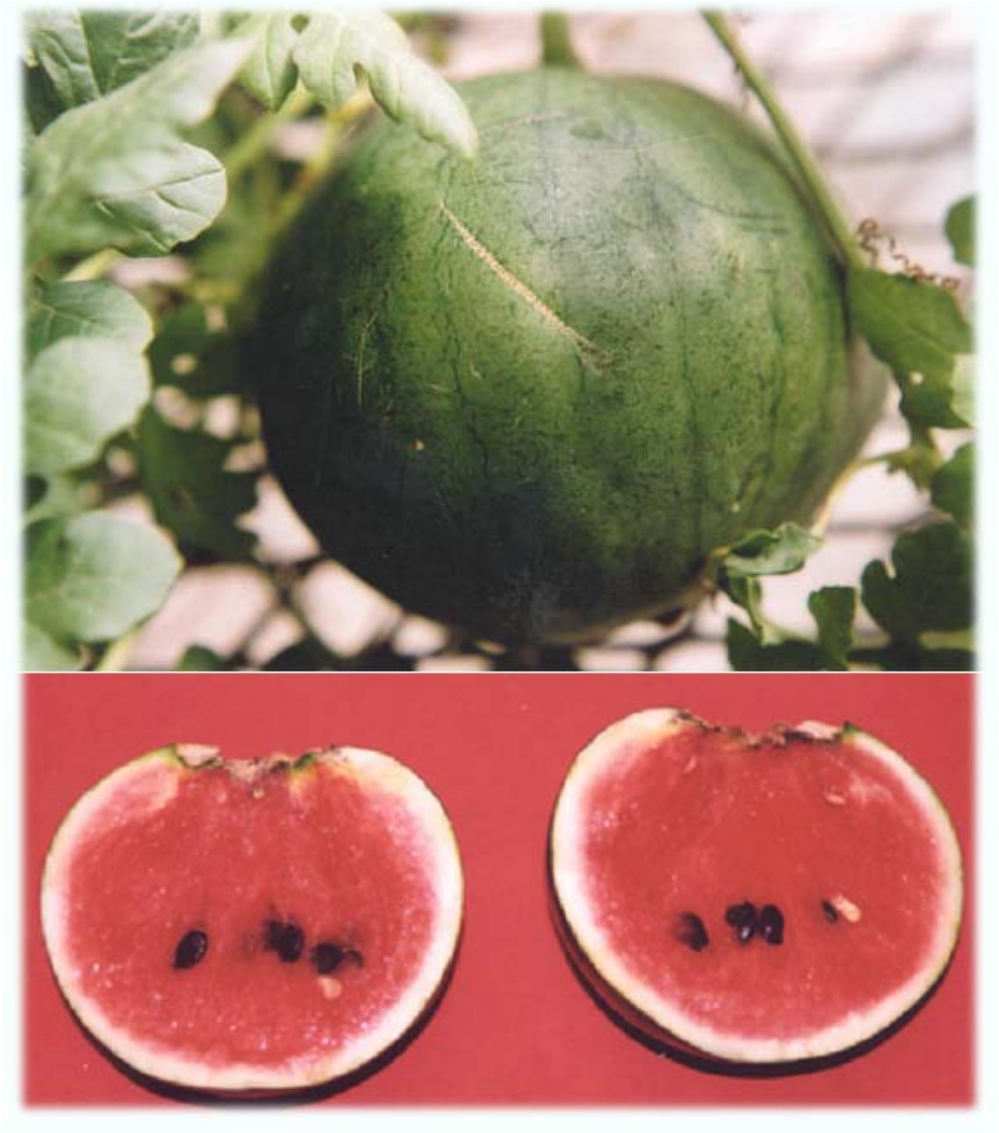

Figure 8. Ro-transgenic watermelon fruit of the cv. Giza1 containing the *cp* gene of ZYMV-E strain grown under biocontainment conditions. ***Note***: this line will be subjected to evaluation of its virus resistance to ZYMV-E under greenhouse and/or field conditions in the summer season, 2002.

Objective 5: Evaluation of the multiple resistant squash, melon and cucumber lines obtained from Cornell University under greenhouse and/or field conditions

Prof. Dr. El-Downey recommended a few lines for evaluation of their virus resistance under AGERI conditions (greenhouse and field). These lines were previously evaluated for their virus resistance under small-scale contained open field conditions at Sids Experimental Station. The experimental results showed high sensitivity of the tested seeds to salt stress in AGERI's soil combined with low germination percentage (20-25%). On the other hand, results showed good fruit quality of squash and cantaloupe lines cultivated under field conditions at Sids Experimental Station (Figures 9 and 10) and no viral symptoms after 12 week from planting were observed.

Figure 9. Production of multiple resistant squash fruits obtained from Cornell University under field conditions at Sids Experimental Station. ***Note***: These lines did not subject to any viral mechanical inoculation treatment.

6
1

5

Figure 10. Production of multiple resistant cantaloupe fruits (different types) obtained from Cornell University under field conditions at Sids Experimental Station. ***Note***: These lines were not subject to any viral mechanical inoculation treatment.

Discussion/Implications

The field trials conducted at different locations, Giza (AGERI), Beni Suef (Sids), and Kalubia (El-Qanter) have verified that the transgenic squash line Eskandarani is highly tolerant to ZYMV-E strain infection while maintaining high yield and superior marketable fruit quality. Therefore, this was followed by cross- pollination with those lines, which contain the *cp* gene of the most serious strain of ZYMV (ZYMV-E) for production of hybrid transgenic seeds for commercialization. The greenhouse trials conducted for melon were completed and the obtained lines will be subjected to field evaluation as carried out for squash. In the case of watermelon, the introduction of the *cp* gene of ZYMV-E strain into the cv. Giza 1 of watermelon was successful. The transformed explants were successfully acclimatized followed by self-pollination of the Ro-transgenic lines.

Highlights of significant achievements

- Production of hybrid squash lines tolerant to ZYMV-E strain by cross pollination between lines containing the *cp* gene of ZYMV-CT strain and others contain the *cp* gene of ZYMV-E strain.
- Evaluation of highly ZYMV-tolerant melon lines (R4-transgenic plants) containing the *cp* gene of ZYMV-CT strain under plastic greenhouse conditions.
- Introducing the *cp* gene of ZYMV-E strain into the cv. Giza 1 of watermelon followed by producing the Ro-transgenic line(s) and R1 seeds.
- Evaluation of virus resistance in the selected multiple virus squash, melon and cucumber lines obtained from Cornell University under greenhouse and field conditions.

Acknowledgments

The research team would like to express their gratitude to all the staff of BioComputing Unit and greenhouse facilities for their sincere help during this work.

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Potato Transformation for Development of Potato Tuber Moth Resistance

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Overall Project Goal

The overall objective of the project is to test resistance management theories with respect to maintaining effectiveness of host plant resistance in potato against potato tuber moth. The specific objectives are to:

1. Continue genetic engineering development with emphasis on genes available for commercial development.
2. Continue transformations with new gene/vectors for varieties important to Egypt and South Africa.
3. Examine the individual foliar expression levels of new transformations in lab and field tests.
4. Evaluate the effectiveness of individual and combined resistance factors under laboratory experiments.
5. Develop linkages with US companies and Egyptian and South African seed companies to promote the commercialization of desired potato lines.
6. Evaluate different management strategies for maintenance of resistant potato varieties and their integration into IPM systems.

Importance of Problem

The cultivated potato, *Solanum tuberosum* is the fourth most important food crop in the world. A potato crop produces, on average, more food energy and protein than cereals. Not only is the potato an important food for the fresh market, but also it is the raw material for french fry, chipping, and starch processing. It is highly productive on a per acre basis and can be grown commercially over many latitudes and elevations in 130 of the world's 167 independent countries.

Potato tuber moth, *Phthorimaea operculella* Zeller, is the most serious insect pest of potatoes in sub- and tropical latitudes. Insecticide use is the most common means of potato tuber moth control in both field and storage. Twelve to twenty insecticide applications are commonly used to control

potato tuber moth during the growing season, three to four insecticide sprays or dusts are often applied for potato tuber moth control in storage with the last application within 1 week of marketing (R. A. El-Bedewy, pers. comm.).

Bacillus thuringiensis is an aerobic, gram-positive, soil bacterium that accumulates high levels of insecticidal crystal proteins during sporulation (McGaughey and Whalon, 1992). These crystalline protein inclusions, or δ-endotoxins, are the principal active ingredients in Bt formulations currently in use (McGaughey and Whalon, 1992). The advantage of the Bt toxin over conventional chemical insecticides is host specificity. Bt bacteria produce insecticidal crystal proteins that are encoded by single genes and transgenic plants expressing the Bt-gene are the most effective means to deliver Bt-based insecticides. The major advantages to this delivery system are increased efficacy, reduced application costs, and minimal scouting needs compared to conventional insecticide sprays. The efficacy of codon-modified Bt genes such as *Bt-cry1* and *Bt-cry3A* is greater than efficacy of the wild type Bt genes in crop plants (Perlak et al., 1991). Many strategies for managing Bt crops have been discussed including the following: 1) high level of a single toxin; 2) mixture of non-resistant and resistant plants in the field; 3) the use of low level toxins and biocontrol agents; 4) toxins deployed sequentially; 5) pyramiding multiple toxins (Gould, 1986).

The expression of Bt genes in plants poses the risk of the insect adapting to the protein. If we can learn to manage this risk in potato, we can exploit the ecologically safe strategy of Bt-deployment. Theories of insecticide resistance management have potentially broad application to managing host plant resistance factors (McGaughey and Whalon, 1992). Most insecticide resistance management depends on alternation of chemicals, allowing refugia for susceptible insects, and use of non-chemical mortality factors (Georgiou and Taylor, 1986). Computer models describing insect adaptation to transgenic plants use inheritance characteristics of the resistant gene, seed mixtures and refugia to predict occurrence of insect resistance. Many resistance management systems are based on population genetic models, the validity of which is only as good as the validity of the assumptions that make up the model as empirical data are scarce or lacking (Tabashnik, 1994). We hypothesize that these same approaches can be used to maintain the effectiveness of host plant resistance factors.

Project Background

This project was started in 1992. Initially, training was a key component of the project. Scientists from AGERI visited MSU to learn techniques about vector construction, potato transformation, field-testing of transgenic potatoes, potato tuber moth bioassays, intellectual property rights and biosafety.

Rational for Approach

The *Bt-cry5* toxin gene has been codon modified to increase its expression level in the plant and transferred into potato. Douches et al. (1998) used different *Bt-cry5* constructs for engineering the potato to express high level of Bt toxin effective against potato tuber moth. Some of these transgenic lines were evaluated to determine the foliar resistance to potato tuber moth (Westedt et al. 1998). Li et al. (1999) produced a series of *Bt-cry5* and transformed the cultivar Spunta. Foliage bioassays with potato tuber moth revealed high expression levels. For these transgenic lines to be

commercially successful in reducing potato tuber moth damage, we must assess the tuber resistance from the field and storage. The objectives of this study are to evaluate field-grown *Bt-cry5* transgenic potato tubers for their resistance to potato tuber moth. In addition, to evaluate of 11-12 month old stored transgenic tubers to determine the mortality efficiency of the expressed *Bt-cry5* toxin.

These transgenic potatoes will reduce the use of insecticides in Egypt and South Africa thus allowing for the production of a safer product for human consumption. Additionally, potato production with less insecticide use will have less impact on the insecticide contamination of the environment.

Previous Research

Initially, transformations with the *Bt-cryla(c)* wild type gene were performed using cv. 'FL1607' as a model system (Hudy 1997). Yadav and Sticklen (1995) developed a genotype independent potato leaf disk regeneration protocol. This regeneration protocol was adapted to our Agrobacterium mediated transformation protocol (Douches et al. 1998). The first Cry5-Bt construct (with the GUS gene fused to the Cry5-Bt gene) was used in transformations with cvs. 'Lemhi Russet', 'Atlantic', L235-4 (glandular trichome line), and USDA8380-1 (foliar leptine line) (Westedt et al. 1998). The *Bt-cry5* constructs that differ in the promoter (CaMV 35S, Gelvin super promoter and patatin promoter) were transformed into cv. 'Spunta' (Li et al. 1998a). The *Bt-cry5-PVYcp* gene construct was also transformed into 'Spunta' (Li et al. 1998b). Spunta is the most important cultivar grown in Egypt and is cultivar used by resource poor farmers in South Africa, while Atlantic is a desired chip-processing cultivar. Other constructs that have the GUS gene removed have been used for transformation. A sample of the *Bt-cry1* and *Bt-cry5* transgenic lines was transferred to AGERI as tissue culture plantlets for greenhouse testing.

Detached leaf bioassays are used to determine the level of host plant resistance to potato tuber moth. Various potato lines were screened for natural resistance to potato tuber moth. All PCR-positive Bt transgenic lines developed from this project were screened for resistance to PTM. In addition, a series of other transgenes were evaluated but had no effect upon PTM mortality. We also obtained a number of synthetic *Bt-cry1a*-transgenic potato lines from the USDA to test; these lines gave strong control of the tuber. The most promising lines from the detached leaf tests were also advanced to laboratory tuber bioassays. Tuber bioassays identified a series of *Bt-cry5*-Spunta and *Bt-cry5/PVY*-Spunta with high levels of potato tuber moth mortality (Li et al 1998a). Other *Bt-cry5*-transgenic lines (Atlantic, Lemhi Russet and L235-4) were less effective in controlling the tuber moth, but were significantly different from the non-transgenic cultivars.

Agronomic evaluation of the *Bt*-transgenic potato lines was initiated in Michigan in 1994. Yearly agronomic evaluations were have been conducted at this location and the trial size has increased to accommodate the number of Bt-lines being tested. These trials have shown that many of the *Bt*-transgenic lines performed similar to their non-transgenic cultivar. These trials also served as a training site for the AGERI scientists for biosafety and potato varietal assessment. With agronomic evaluations established in Michigan, seed tubers were produced for Egyptian field-testing.

The first field test of genetically engineered potatoes in Egypt occurred in January 1997 at AGERI after the Egyptian biosafety regulations were established. The purpose of this trial was to evaluate

an array of Bt-transgenic potato lines for field resistance to potato tuber moth. Fourteen lines were evaluated for foliar and tuber damage. To apply greater tuber moth pressure, the field was artificially inoculated during the season. Foliar mining was as high as 38 mines per 10 untreated plants, whereas the Bt-lines had as few as low as 1 mine per 10 plants. Non-transgenic tuber infestation was 80-92% (severe level of infection). In contrast, some of the Bt-transgenic lines had as little as 38% infection of the tubers. These results were very promising and expanded field trials were established for 1998 in Egypt. In February, AGERI trial was repeated and an insect and an agronomic trial were planted at the CIP Potato Research Station (located in the delta potato-producing region).

Field trials were conducted in Egypt in 1999, 2000 and 2001 at AGERI and CIP-Egypt. The results show excellent control of PTM from several lines including Spunta G2 and Spunta G3 that had virtually no potato tuber moth infestation compared to the non-transformed control lines in our CIP field trial. Storage experiments were conducted with the harvested potatoes and again the Spunta G2 and Spunta G3 lines had minimal infestation for nearly 3 months in storage.

Specific Objectives

1. Continue genetic engineering development with emphasis on genes available for commercial development.
2. Continue transformations with new gene/vectors for varieties important to Egypt and South Africa.
3. Examine the individual foliar expression levels of new transformations in lab and field tests.
4. Evaluate the effectiveness of individual and combined resistance factors under laboratory experiments.
5. Develop linkages with US companies and Egyptian and South African seed companies to promote the commercialization of desired potato lines.
6. Evaluate different management strategies for maintenance of resistant potato varieties and their integration into IPM systems.

Research Progress

A 2001 field trial was conducted in Egypt at CIP-Egypt. The results show excellent control of PTM from our transgenic lines (Fig. 1). Storage experiments were conducted with the harvested potatoes and we are waiting for the results of these tests.

Seed production increases for year 2001 field trials in Egypt and South Africa were made at the MSU Potato Research Center, Montcalm Co., MI. Additionally, greenhouse tubers were produced by the private sector for field trials. We have 2000 seed pieces of various 'Spunta' lines (Spunta G2, Spunta G3, Spunta S1, Spunta S4 and Spunta 6a-3 [PVYcp/Cry5]). Our plans are to test these lines in Egypt and South Africa for seed increase and test on commercial farms in South Africa in year August 2002. Planting these lines on commercial farms will allow growers to see the benefits of these lines and hasten commercialization.

Agronomic trials were conducted in Michigan testing the *Bt-c*ry5-Bt Spunta lines. All lines were comparable to the non-transformed control.

New constructs using four different promoters for *Bt-cry5* expression were developed. The different promoters include CaMV35s, Gelvin super promoter (GSP), Patatin, and Ubiquintin3. The Ubiquintin3 promoter was developed by USDA/ARS and thus eliminates several IPR restraints. This promoter is being used to design a freedom-to-operate vector, which may hasten commercialization of our transformed potato lines. We are currently conducting detached-leaf feeding bioassays in the lab with 'Lady Rosetta' and 'Atlantic' lines transformed with the different constructs. We are also conducting experiments to use the MAT vector system that removes the selectable marker from the transgenic line during the transformation/regeneration steps.

New constructs using the same four promoters have been developed for codon-modified *Bt-c*ry1Ac (J. Kemp, NMSU) expression. Potato transformation will begin in the near future.

Discussion/Implications

Potato transformation with our different promoters and new genes are showing excellent control of PTM. Seed increases of these lines will allow for test on grower's farms in Egypt and field tests in South Africa. This is a important step to commercialization as it allows the producer the opportunity to observe first hand the benefits of the product. Transforming 'Atlantic' and "Lady Rosetta" is also an important step towards commercialization in Egypt as these varieties are very important in the Egyptian chip industry. We are also targeting Mexico for field-testing and commercialization (We have had some interest from Frito-Lay, Inc.)

Highlights

As stated above and additionally, the previous field and future field tests conducted in Egypt. We have also planted a field trial at ARC-Roodeplaat in South Africa.

Publications

Douches.D.S, W. Li, K. Zarka, J. Coombs, W. Pett, E. Grafius and T. El-Nasr. 2001. Development of Bt-cry5 Insect Resistant Potato Lines Spunta-G2 and Spunta-G3. HortSci. (in press).

Presentations

Douches, DS: NCR-84 Potato Breeding and Genetics Technical Committee, Dec 2001, Minneapolis, MN.

ABSP TAC, June 2001, East Lansing, MI.

Travel

W. Pett to AGERI January 2001

Francisco Santos to AGERI May, 2001

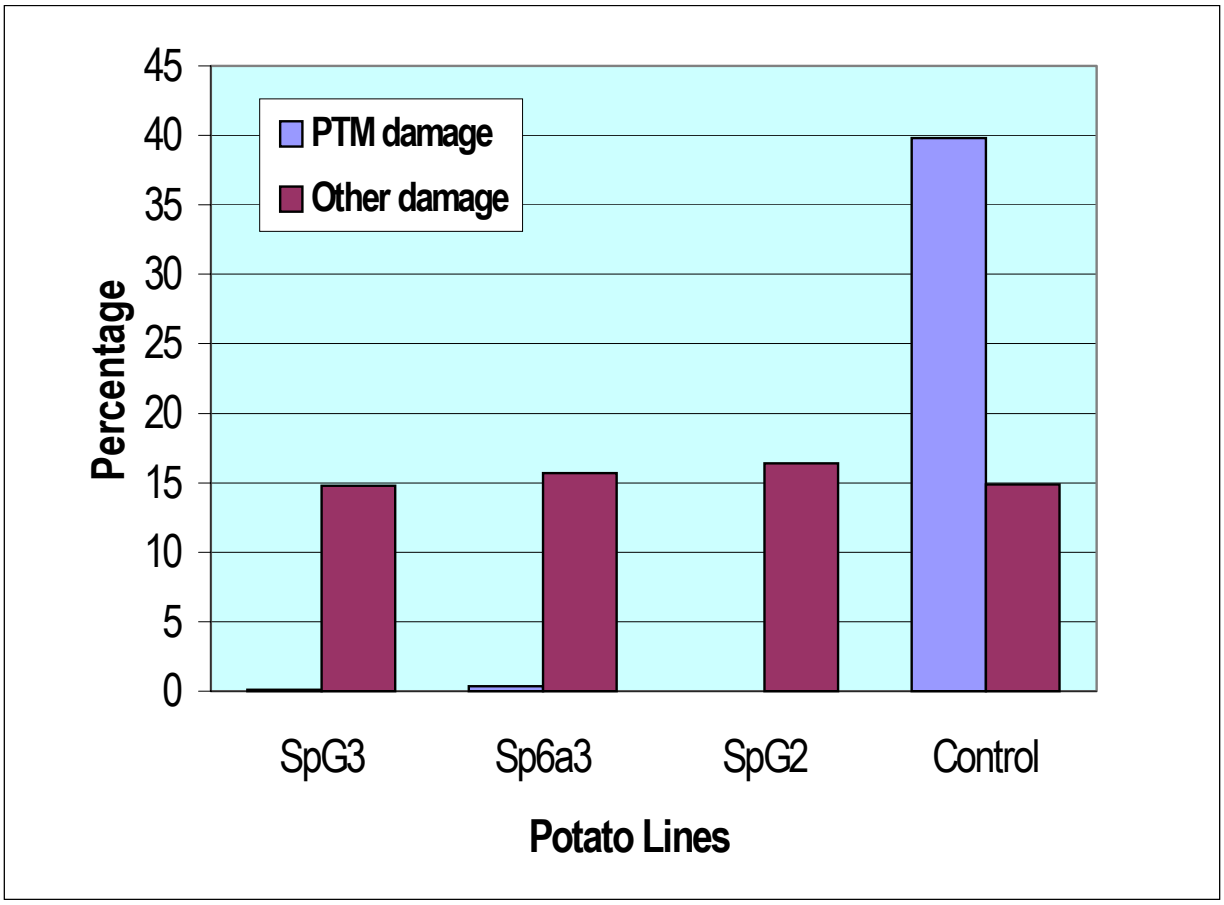

Figure 1. The percentage of potato tubers harvested at CIP, Egypt that were damaged by PTM and the percentage of tubers damaged by non-PTM insects (cutworms and mole crickets) or other factors (sun exposure or mechanical).

Managing Natural and Engineered Resistance in Potato to Potato Tuber Moth

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Background

Potato (*Solanum tuberosum* L.) is the second important vegetable crop after tomato in Egypt. The area of potato under production has reached 292,000 hectare/year over three seasons (i.e. winter, spring, and summer). The total production is around 2.5 million tons annually with the winter season crop used mainly for export. Egypt exports 250,000 tons to Europe and the Arab countries. The yield is affected by infestation with potato tuber moth *Phthorimaea operculella* Zeller (PTM). The infestation decreases the yield by 20 to 40 % according to the pressure of the insect and the growing season moreover, it effects tuber quality .The insect attacks potato plants in two ways: by mining the foliage and feeding on tubers as well. Therefore, it is an important pest both in field and storage.

Objectives

The project goal is to develop insect resistant potato lines via genetic engineering mainly against potato tuber moth PTM, *Phthorimaea operculella*. The strategy for resistance is based on transformation with Bt toxin gene.

1. Develop and standardize bioassay procedure for testing Bt varieties against PTM and other insect pests.
2. Improve regeneration and transformation system for Egyptian potato cultivars.
3. Evaluate Bt-transgenic lines developed through the collaborative project activity under Egyptian field conditions.

Research Achievements

Field trials:

ABSP project provided Bt. transgenic potato lines to test their resistance to PTM in field trials in Egypt after obtaining clearance from USAID, Egyptian National Bio-safety Committee (NBC) and CIP-Lima. One trial was carried out at AGERI Experimental plot and the other was conducted at CIP station located in the middle of the Delta (potato growing area) for evaluation of the level of resistance to potato tuber moth (PTM).

Field trials conducted on Feb. 2001 involved transgenic lines with different Bt -constructs and various gene promoters.

This year we focused on three lines derived from Spunta cultivar (SPG2, SPG3 and Sp-6A-3). Potato tubers were planted in a randomized complete block design with 3 replications. Treatments included transgenic with various modified and non-modified gene constructs. The experiment at CIP site was conducted under natural infestation condition whereas in AGERI field plots were infested artificially. The insect damage on foliage was evaluated 3 times during the growing season. The trials were harvested on June 2001 and tubers were examined for PTM damage.

Transformed potato tubers were harvested at AGERI and CIP Egypt. Results revealed that selected lines of Spunta have confirmed high level of resistance to PTM compared to control under field conditions.

Storage Experiment

Healthy tubers presenting the transgenic and non-transgenic lines were stored in a traditional storage facility (Nawalla), which has no cooling system. Tuber damage was examined during the storage period. Three inspections were carried out at one-month interval. Percentage of infested tubers was recorded in Table (1). Data revealed that the control line i.e. Spunta is more susceptible to infestation compared to transgenic lines.

Table 1: Percentage of tubers infested with PTM under Nawalla conditions

Lines	% Infestation		
	1st inspection	2nd inspection	3rd inspection
Sp (Control)	80	100	100
SP G2	1.33	1.33	1.36
SP G3	2.66	3.66	2.5
SP-6A-3	3.66	4.33	1.8

Bioassay of PTM

This experiment was conducted to measure and to reconfirm the efficiency of transformed potato lines under heavy PTM infestation. Healthy transgenic potato lines that were saved from previous field trials were subjected to 50 first instar of PTM larvae per tuber with three replications. The infested tubers were incubated in insect proof containers till emergence of the moths. Number of

non –transgenic tubers from different lines was saved as control. After 28 days, number of emerging PTM moths were counted (Table 2) and compared to control . Data indicated that even under very high insect infestation (50 larvae/tuber) the gene expression was working perfectly specially in Spunta transgenic lines. Data shows that Spunta lines viz. Sp G2, Sp G3 and Sp-6A-3 were the most promising lines.

Table 2: Inhibition percentage of the PTM adult moth emergence on transgenic and non-transgenic potato tubers.

Lines	Construct	No. of adult moths*	% Moth inhibition from emergence (I)
Spunta (Control)	-	41	0
Diamont (Control)	-	43	0
Desiree (Control)	-	24	0
SpG2	Bt-CryV	0	100
SpG3	Bt-CryV	0	100
Sp-6A-3	Bt-CryV cp-PVY	0	100

** Emerged from 60 larvae after 4 weeks*

$$I = \frac{\text{Number of adult moth of control - Number of adult moth of lines}}{\text{Number of adult moth of control}} \times 100$$

Laboratory test

The stability of the genomic structure for transgenic versus non-transgenic lines was studied using RAPD analysis. Ten different random primers were used in RAPD test (Fig 1). Results revealed no-variation between transgenic potato lines Sp G2, Sp G3 and Sp-6A-3 resistant to PTM and non-transgenic lines.

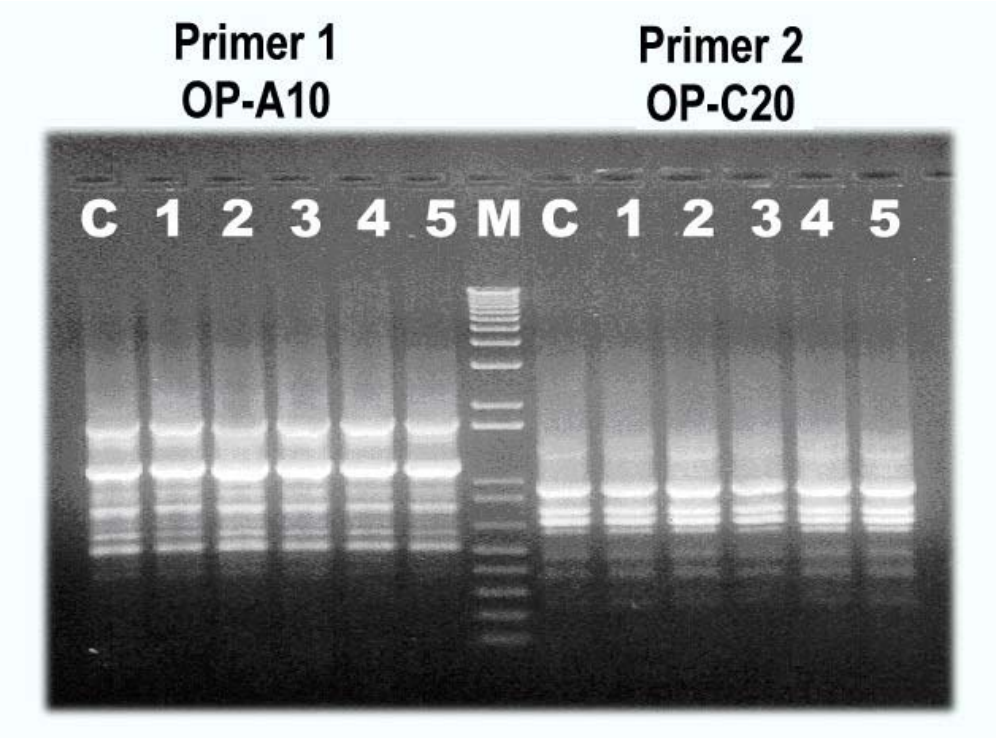

Fig (1): 1% agarose gel electrophoresis for transgenic and non-transgenic potato lines using random primers.

Lanes (1&8): Non-transgenic potato line (Control)
Lanes (2 to 6) and (9 to 13): Transgenic potato lines
Lane (M): Marker

Regeneration Studies

Comparison between two regeneration protocols was carried out on different potato cultivars (Spunta, Desiree and Diamond) to select the most efficient regeneration system. Protocol No. 1: contained MS salts medium+1mg/IBA+1mg/IIAA+10 mg/l GA_3, While protocol No.2: contained MS salts medium+1mg/NAA+1mg/l Zeatien. Generally, protocol No 1 produced higher regeneration percentage compared to protocol No.2. This protocol could be applied to all tested potato cultivars.

Conclusion

It could be concluded that the regeneration and transformation systems are well established at AGERI for most of the important potato cultivars on the Egyptian market. Gene transfer is conducted through *Agrobacterium* -mediated transformation techniques.

The clearance of transgenic potato for food and feed safety and environmental assessment is still required by the Bio-safety Committee before taking steps towards the registration of Bt. transgenic lines for local commercial release. The Technology Transfer and Intellectual Property office at AGERI will help out with all the steps necessary for the commercialization of the transgenic product.

The PTM transgenic potato lines can help to cut down the amount of seed potato imported annually from abroad (65000 ton).

We are planning to continue field-testing of PTM transgenic lines in 2002 to confirm the stability of genes and the level of resistance in collaboration with MSU potato team.

Maize Transformation for Development of Stem Borer Resistance

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Overall Project Goals and Objectives

1. Establishment of efficient regeneration and transformation system(s) for Egyptian elite maize germplasm.
2. Use the above system(s) to introduce AGERI propriety owned Bt gene(s) to confer resistance to stem borers.
3. Evaluation of four novel constitutive maize promoters (outcome of Pioneer/AGERI collaboration) in driving the transient and stable expression of the GUS reporter gene in commercial maize hybrids. This will lead to stability in yield potential of the hybrids due to insect resistance of the transgenic plants. In addition to reducing pesticide application, which will have a positive impact on the environment and production costs.

Project Importance

Maize as one of the major cereal crops in Egypt, it is cultivated in an area of about 1.8 million acres. Corn borers (*Sesamia cretica, Ostrinia nubilalis and Chilo agamnon*) are serious insect pests in most of the corn growing area of Egypt and are responsible for significant loss of yield (around 40%). Application of chemical pesticides has been the only control measure taken against these insects. This project proposes to introduce Bt gene(s) that are known to code for proteins that are lethal to lepidopteran species into Egyptian commercial maize hybrids.

Project Background

Among the insect pests that infest corn plants are the stem borers of which *Sesamia cretica* causes most damage as cultural practices, mainly sowing corn fields during mid-May to mid-June, has led to minimizing the infestation with *Ostrinia nubilalis* (European corn borer) and restricting its damage

to late plantings (July). Application of chemical pesticides has been the only control measure taken against these insects. Plant biotechnology has introduced new tools to control damage from insect pests. During the past few years, the introduction of Bt gene into maize and other crops has become an established procedure (Kozeil *et al* 1993 and Bohorova *et al*., 1999). Therefore, we are aiming at the introduction of Bt gene into elite Egyptian maize lines using the bombardment system.

Previous Research Results

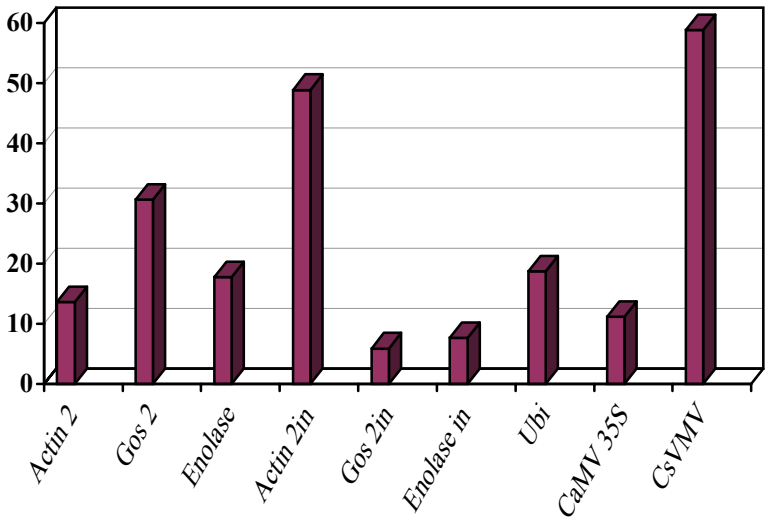
1. Different Egyptian maize inbred lines were screened for their regeneration ability from immature embryo culture & shoot tips using different media.
2. Modification in media composition resulted in improvement of regeneration ability in both systems.
3. Optimum concentration of the selective herbicide agent (Bialaphos) was identified according to the kill curve studies.
4. Transformation was carried out using different bombardment parameters and optimum conditions were identified with both types of explants.
5. Transient and stable expression of GUS gene was detected in transformed calli, shoot clumps and roots respectively.
6. PAT activity was assayed by painting leaves of transformed plants with the herbicide BASTA (1% solution).
7. The integration of transformed genes was confirmed by PCR and Southern blot analyses.
8. The output from one of the training programs at Pioneer resulted in the isolation of four novel constitutive maize promoters (Actin-2, enolase, Gos-2 and L41). The joint ownership of these promoters will give an advantage to AGERI when commercializing a transgenic crop by having proprietary over a vital component of the gene construct.

Research progress

1. Transformation experiments have been carried out using the biolistic gun for the reconfirmation of the transient GUS gene expression driven by the novel maize promoters (Actin-2, enolase, Gos-2 and L41) in maize immature embryos (as a monocot plant) and in tomato leaflets (as a dicotyledenous plant). The expression was compared with that driven by the ubiquitin promoter.
2. Results shown in Fig.(1-a) revealed that the highest expression of the GUS gene in maize was obtained by the Gos-2 and enolase promoters in the presence of intron. Moreover, the results revealed that the intronless Gos-2 promoter was less efficient than the Ubi promoter (control) in driving the GUS expression, however, this level of expression could be sufficient in the transformation of different genes.
3. In case of tomato transformation the results revealed that the Actin-2 promoter with intron and the Gos-2 promoter without intron revealed the highest GUS expression in comparison

to the CsVMV and CaMV-35S promoters as control (Fig. 1-b).

4. Experiments have been carried out to evaluate the novel promoters for the stable expression of the GUS gene in maize plants.
5. Plasmid construction is being continued for constructs that contain these novel promoters and GUS gene with the bar gene (with or without intron). The efficiency of these promoters in stable transformation of the bar gene will be tested.
6. The highly recalcitrant elite maize line Sd7 has been crossed with the American line A188 which is known to produce embryogenic calli. The regeneration frequency have been significantly improved it reached 62.33% in comparison to that of Sd7 (22.33%).



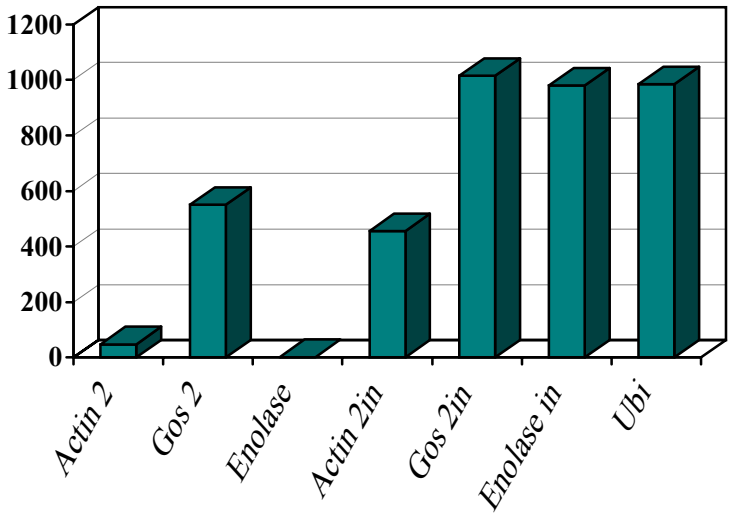


Fig. (1): (a) Average number of GUS blue spots in transformed maize embryos. (b) Average number of GUS blue spots in tomato leaflets.

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Assem S. K., H. A. El-Itriby, E. H. A. Hussein and M.A. Madkour. Development of efficient regeneration and transformation systems in Egyptian maize lines. *A Poster presented in The 6th International Congress of Plant Molecular Biology held in Quebec, Canada*, 18-23 July 2000.

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El-Itriby H. A., S. K. Assem, E. H. A. Hussein, and M. A. Madkour (2002). Regeneration and transformation of Egyptian maize inbred lines *via* immature embryo culture and biolistic particle delivery system. (*Under Publication in The Arab Journal of Biotechnology*).

High Beta-Carotene Mustard Project

Lead Principal Investigators

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Overall Project Goal

To develop high beta-carotene mustard ('golden mustard') for potential deployment in a food based approach to reduce vitamin A deficiency in India. This first phase of the project mainly involves the transfer and use of technology already developed for temperate varieties of canola into Indian varieties of mustard and the subsequent varietal testing under Indian laboratory and field conditions to assess agronomic performance and nutritional value.

Background

Vitamin A deficiency is endemic in the developing world, with the result that nearly 250 million people suffer chronic disease and even death due to lack of dietary access for this important micronutrient. Vitamin A malnutrition is prevalent in India. Most at risk are young children and women of childbearing age. Estimates are that 5-7% of Indian children are at various levels of Vitamin A malnutrition. Developing countries, often aided by donor programs, have attempted to address Vitamin A malnutrition through supplementation and fortification programs. While successful in some developing countries that have adopted a sustained effort, non-food based methods of dietary supplementation have yielded only sporadic results. The new techniques of biotechnology offer an effective timely and scientifically feasible means to enhance the beta-carotene or pro-vitamin A content of common foods.

Beta-carotene is a precursor to vitamin A. The human body converts beta-carotene efficiently to vitamin A. In contrast to vitamin A, high doses of beta-carotene are not toxic to humans. This project is to develop Indian varieties of mustard high in beta-carotene using plant biotechnology. Monsanto has been successful in developing canola varieties high in beta-carotene via plant biotechnology. As part of its commitment to sharing knowledge and technologies to improve subsistence crops and benefit human health in developing countries, Monsanto has licensed, royalty-free, use of its carotenoid technology for the development of high beta-carotene mustard. The Tata Energy Research Institute (TERI), with the assistance of Michigan State University's Agricultural Biotechnology Support Program, will develop mustard varieties expressing adequate levels of beta-carotene to reduce vitamin A deficiency in India. This first phase of the project entails:

1. The transfer of technology developed for temperate varieties of canola into Indian varieties of mustard (*Brassica. juncea*).
2. The subsequent varietal testing under Indian laboratory and field conditions to assess agronomic performance and nutritional value.
3. The training of Indian scientists in the various techniques and disciplines required in

developing high beta-carotene mustard as a viable solution to vitamin A malnutrition in India.

4. The conduct of preliminary studies to evaluate the socio-economic use patterns associated with high beta-carotene mustard oil as a component of the Indian diet, especially in relation to vulnerable segments of society.
5. A preliminary assessment of intervention mechanisms that might be suitable for the introduction of beta-carotene enhanced mustard.
6. Organize workshops to increase awareness about the utility of a food-based, biotechnology approach for nutritional enhancement and to address concerns/issues among various stakeholders and also to understand the requirements of stakeholders and constraints if any for commercialization.

Upon successful completion of this work, which is expected to result in the development of a suitable variety of locally adapted Indian mustard with enhanced beta-carotene content, a follow-on program (phase II) would be initiated within India by the relevant scientific and development institutions, to more comprehensively evaluate and initiate the use of this variety in a pilot, food-based, intervention scheme to address Vitamin A deficiency.

Project Partners

Monsanto Company: Monsanto Company is a leading provider of agricultural solutions to growers worldwide. Monsanto's employees provide top-quality, cost-effective and integrated approaches to help farmers improve their productivity and produce better quality foods. Monsanto is also committed to sharing knowledge and technology to advance science and understanding, improve agriculture and the environment, improve subsistence crops, and help smallholder farmers in developing countries.

Tata Energy Research Institute (TERI): Established in 1974, TERI is an autonomous, not-for-profit, research institute with headquarters located in New Delhi, India. TERI is involved in a broad range of research activities, including those related to biotechnology, renewable energy, forestry and policy analysis. Given the importance of edible oil to the country, a program on Brassica was initiated in the late 1980s. The research program embraces both applied and basic aspects, and concentrates mainly on the improvement in quantity and quality of oil production by combining the molecular and conventional methods of genetic manipulation. Research has been conducted on the following areas: (1) increasing productivity through hybrid seed production; (2) incorporating useful agronomic traits through wide hybridization and embryo rescue; (3) developing rapeseed-mustard lines with improved nutritional quality such as low erucic acid, high oleic acid and low glucosinolate; and (4) studying the genome organization of Brassicas for developing species-specific probes to understand the phylogenetic relationships of different Brassica species and use of molecular markers for varietal typing.

ABSP: This USAID-funded project based at Michigan State University was established in 1991 to facilitate the transfer of proprietary biotechnology practices and products to developing country institutions (both public and private) to address agricultural production constraints of local relevance. The program has also sponsored a significant biotechnology capacity building

effort focused on, among other things, biosafety and regulatory capacity building to establish legislative guidelines and protocols for product review in developing countries. ABSP activities and interventions have resulted in the establishment of biosafety review frameworks in Egypt, Kenya and Indonesia. ABSP also has a demonstrated ability to programmatically and fiscally manage complex projects involving diverse partners and it is envisioned that they will play a similar role for this program.

National Institute of Nutrition (Hyderabad, India): NIN is affiliated with Government of India. NIN has agreed to provide assistance in conducting preliminary nutritional analysis and other product evaluation studies of the new mustard varieties and modified oil.

Department of Biotechnology (DBT, New Delhi, India): DBT is responsible for conducting regulatory reviews of the genetically modified varieties and for protocol development/monitoring for associated food studies and field trials in conjunction with NIN and the Indian Council of Agriculture Research.

Integrated Child Development Scheme (ICDS): The ICDS represents a potential vehicle for the delivery of the enriched beta-carotene mustard oil resource to poor children and mothers. ICDS is a Government of India (GOI)-sponsored program for the development of children below age six. The program was launched on October 2, 1975 in 33 districts in India. The program is designed for low-income children and children from families below the poverty line in rural and tribal areas and urban slums. Over the years, women's health, especially that of pregnant and lactating women has been added to the scope of the ICDS. Presently, the program reaches nearly all areas of India. The focal point of the ICDS is the Anganwadi Centers (AWC), used to deliver ICDS services to beneficiaries. The program, while sponsored by the Central government, limits the government role to the provision of funds. Program operation is overseen at the state level by the State Director. Food for supplementation is supplied either by NGOs working with the ICDS program or state government approved contractors. Foods included are "ready-to-eat", "ready-to-cook" or basic ingredients for cooking on site at AWC centers.

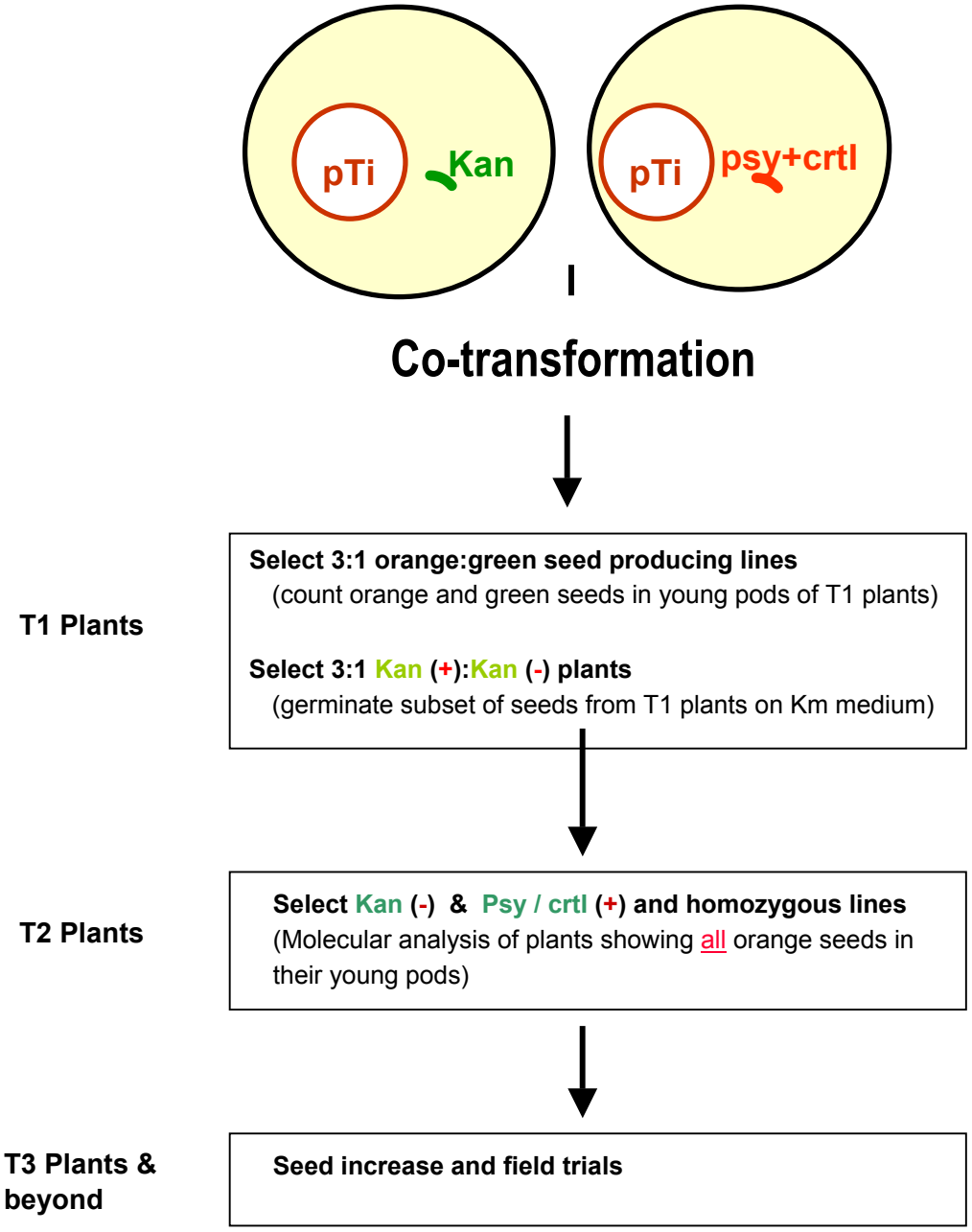
Research Progress

In plants, beta-carotene biosynthesis occurs in plastids. The enzyme that catalyzes the first committed step in carotenoid biosynthesis is phytoene synthase. Elevated expression of phytoene synthase in plastids of developing embryos of *Brassica napus* (canola) had been previously demonstrated to result in ca. 50-fold increase in the level of beta-carotene, in addition to the increase in other nutritionally useful carotenoids in mature seeds. When oil is extracted from such seeds a large proportion of these carotenoids are partitioned into the oil fraction.

Mustard (*Brassica juncea*) is widely grown in India. There are several *B.juncea* mustard varieties grown in northern parts of India; among which three varieties, Varuna, Pusa Bold and RH30 are the most popular ones. These three genotypes (Varuna, Pusa Bold and RH30) were thus selected by TERI for transformation, who also supplied the necessary quantities of mustard seeds. Compared to canola (*Brassica napus*), the transformability of *Brassica juncea* is very poor. The genes coding for phytoene synthase (*Psy*) and phytoene desaturase (*CrtI*), both

driven by a seed specific promoter, napin, were introduced into Indian mustard varieties to enhance the beta-carotene levels in their seeds. *Agrobacterium* transformation was used to transfer the genes into mustard. In order to later remove the antibiotic selectable marker gene from the final product, a co-transformation technique (using two T-DNA's) was used. In this approach, the kanamycin resistance selectable marker gene was placed in one of the binary vectors (Kan^R) and the genes of interest (*Psy* and *Crtl*) placed in a second binary vector (Psy-Crtl). Each vector was transferred into a separate *Agrobacterium* stock. *Brassica juncea* explants were co-transformed using the mixture of the above two agrobacterial stocks viz., one having the Kan^R binary and the other having the Psy-Crtl binary (Figure 1).

Figure 1. **Two T-DNA Strategy to Remove Antibiotic Marker**



Out of a total of over 100,000 explants from the three varieties that were infected during 2000-2001 period, fifty viable shoots were recovered. The fifty shoots yielded 8 orange seed-producing lines (seeds turn orange because of the higher carotenoid content), which included the three varieties (Varuna, Pusa Bold and RH30). About 100 T-2 generation plants from each of the eight orange seed producing T1 lines were screened for the absence of antibiotic marker gene. Approximately 25 T2 plants have been found to be free of antibiotic maker gene and homozygous in *Psy* and *Crtl*.

The above technical work on transformation and screening of *Brassica juncea* was carried out at the Monsanto Research Center in Bangalore, India. Seeds from these plants are currently awaiting shipment to TERI for greenhouse and field trials. Meanwhile, TERI is engaged in securing necessary permits for transport of this transgenic mustard seeds to TERI research facilities.

Other Activities

- The TERI scientist, Dr. Ashok Choudhary, was trained in mustard transformation techniques at Monsanto's research facility at Bangalore, India from August 6, to August 20, 2001.
- Dr. Cherian George of Monsanto and Dr. Vibha Dhawan of TERI presented the 'mustard project' at the ABSP's external Board meeting/review at Washington on November 2, 2001.
- Dr. Cherian George and the director of TERI, Dr. Rajendra K. Pachuri visited US Senator Hillary Clinton and Congressman Richard Gephardt and discussed the progress of the 'golden mustard' project in India on June 5, 2001.
- An advisory committee of prominent Indian scientists was formed to guide the project. The committee is scheduled to meet periodically at the TERI facility in New Delhi to set direction and monitor the progress of the project.
- Dr. Cherian George gave a number of seminars on 'golden mustard' and nutritionally enhanced crops during 2001.

Developing Drought And Salinity Tolerant Wheat For Egyptian Agriculture

Lead principal investigators

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Project partners

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Overall project goal

The ultimate goal of this project is to enhance osmotic stress tolerance in Egyptian wheat crop through genetic transformation. This goal can be achieved by over expressing the key regulatory enzymes of the proline biosynthesis and sulfur assimilation pathways. We will directly determine whether elevated levels of proline and active sulfur confer drought and salinity tolerance in wheat. Attempts from the American partner will be made to find gene(s) able to convert proline into proline betaine.

Importance of the problem

To meet the food demands of the growing population, it is extremely important to expand the agricultural system to the drier, less arable lands of Egypt. Therefore, there is a need to identify and characterize stress tolerance (drought and salinity) genes from wild germplasm. Study of the induction and expression of these stress-related genes in wild plants could provide understanding as to how these plants adapt to the arid and semiarid environments. Furthermore, these protocols will provide information for selection of stress tolerance among different wild plant resources. Since wheat is one of the most important pulse crops in Egypt, genetic engineering of stress-tolerance genes to provide varieties, which could grow under stress conditions is the method of choice. Protocols of regeneration and plant transformation are now available at AGERI for Egyptian wheat cultivars, which will be utilized in this concern.

Project background

Constraints against field crops by biotic or abiotic environmental stresses prevent them from achieving their full genetic potential. Water stress caused by drought and salinity is the most important abiotic factor limiting plant growth and crop productivity worldwide. Arable land acreage is limited in Egypt due to the lack of water needed for irrigation. The amount of High Dam water available is only sufficient for two million feddans of cultivated land along the north coast of Egypt. Therefore, the possibility of future irrigation with mixed fresh and seawater raises the need for developing wheat cultivars with increased salt and drought tolerance.

As a monocotyledonous plant, wheat has lagged behind dicotyledonous plants in ease and efficiency of transformation. Thus, a consistent procedure of transformation and regeneration for wheat tissues is needed. In cereals other than rice, it has been very difficult to regenerate fertile plants from protoplasts. Additionally, it has been difficult to maintain regenerable long-term suspension cultures in cereal crops from which transgenic plants can be recovered efficiently. The efficient production of fertile transgenic wheat plants was accomplished by using immature embryos as a target tissue and microprojectile bombardment as the mechanism of DNA delivery. This methodology opened the way to overcome many problems of wheat transformation.

Plants have gained adaptive mechanisms to cope with the effects of water stress through many physiological responses. The best-characterized biochemical response of plant to water stress is the accumulation of organic osmolytes such as proline, polyols such as mannitol. Genetic and transformation data accumulated so far indicate that overproduction of some of these osmolytes results in enhanced tolerance to water stress in some plant models, i.e., arabidopsis, tobacco, etc.

Rationale for approach

Recent advances in wheat transformation technology and molecular and physiological studies of the genes involved in osmotic responses to stress conditions allow us to introduce genes to improve water-stress tolerance in Egyptian wheat cultivars. Transformation experiments using these genes in model plants have shown that increased stress tolerance can be achieved. Results of these experiments also have direct potential for development of salt- and drought-tolerant wheat cultivars for improving wheat productivity in Egypt. Transgenic plants will provide a unique system for understanding plant osmotolerance mechanisms and responses to environmental stresses. The methodology we use for wheat can be considered as a model for other cereal crops in developing tolerant genotypes against abiotic stresses.

Previous research

This project is based on the idea that accumulation of proline, a known osmolyte, can enhance drought and salinity tolerance in crop plants and extend the survivability of crop plants under stress conditions. During the last 7 years, Professor Verma's group (our collaborator) has not only isolated all genes involved in proline biosynthesis, but also demonstrated that *P5CS* is a rate-limiting enzyme in proline synthesis and over expression of this enzyme produces more proline if sufficient nitrogen is available. They have identified a bifunctional enzyme P5CS able to make P5C from glutamate and which limits proline synthesis. Furthermore, they have removed feedback control of this enzyme by proline. This mutagenized enzyme is active up to almost 1M concentration of proline. Recent unpublished data suggest that over-expression of mutagenized *P5CS* can produce more proline in plants. They have obtained a patent on this gene (Patent# 5,344,923; Sept 6, 1994). This gene has been licensed to an Australian company for introducing in forest crops. Therefore, this version of P5CS gene is not available for this project. Instead, we have utilized the wild type version of this gene provided by Dr. Verma.

Rice *HAL2*-like (*RHL*) cDNA has been isolated and characterized by Professor Verma's group for its role in conferring abiotic stress tolerance. This gene supports the growth of cells under high salinity stress. They have further shown that availability of active sulfur was essential to overcome oxidative

stress imposed by salinity and drought stresses. Over expression of this gene in plants results in accumulation of glutathione, which also reduces oxidative as well as salt stress.

At AGERI, we have been successful in increasing regeneration and transformation efficiencies for Egyptian as well as American bread wheats by shortening the selection period, bombarding young immature embryos, using different regimes of hormones and allowing callus to recover for a week after bombardment. We have already finished transforming Egyptian wheats (Giza 163 and Giza 164) with salt-related genes (i.e., *mtlD* and *fructan-accumulating*, respectively) and got promising results regarding tolerance against abiotic stresses.

Specific project objectives:

1. Enhance collaborative relationships that will improve modern genetic technology in Egyptian agricultural research
2. Improve transformation and regeneration efficiencies for Egyptian wheat cultivars
3. Develop different protocols for selecting Egyptian transgenic wheat plants
4. Introduce *P5CS* and *HAL2*-like cDNAs under the control of appropriate promoters in wheat
5. Conduct greenhouse and field performance trials of regenerated transgenic plants
6. Incorporate transgenic plants with improved salt and drought tolerance into ongoing Egyptian breeding programs

Research progress:

Regarding transformation plans at AGERI for this Project, we have been able to finish the task of gene construction one year ago.

A. Transformation and selection:

AGERI has already established a reasonably efficient transformation and regeneration schemes in wheat before starting use of our genes of interest as follows:-

1. Spikes from greenhouse-grown plants were collected 10-18 d post-anthesis. Immature embryos were isolated and placed with the epiblast exposed on callus induction medium as modified for wheat cell culture. Cultures of immature embryos were shown to maintain high frequencies of embryogenic and regenerable calli when scutellum growth is maintained.
2. After 4-7 days in culture, embryo-derived calli were exposed to an MS medium containing mannitol (0.4 M) and bombarded with transformation constructs using the helium-driven DuPont Biolistic Delivery System (Model PDS-1000) with distances of 9 and 13 cm between stopping screen and target cells with one or two shots and pressures of 1100 psi.
3. After a recovery period of one week, bombarded calli were transferred to MS selection medium containing either 0, 3 or 5 mg/l bialaphos.
4. After four weeks of selection, embryogenic calli were transferred to regeneration medium containing the growth regulator thidiazuron (TDZ), which we found to induce optimum levels of regenerability. We used 3 mg/l bialaphos for selection during the regeneration

period.

5. After 2-4 weeks, calli-derived shoots were transferred to hormone-free rooting medium containing 3 mg/l bialaphos.
6. Plantlets with good root formation were transferred to potting mix in the greenhouse.
7. Leaf painting as well as spraying with 1 g/l Basta was done to distinguish transgenic wheat plants at T_0 and T_1 plants, respectively (Figure 1) with a high level of expression of the herbicide-resistance gene (*bar*).

Transformation experiments resulted in the production of two leaf painting-positive putative transgenics with the rice *HAL2*-like (*RHL*) gene as well as one putative transgenic with *P5CS* gene to be tested for both gene presence and expression. More putative transgenics for both genes ought to be accumulated during the winter 2002 season.

Figure 1. T_1 Transgenic progeny with *bar*/*HAL2*-like genes sprayed with the herbicide Basta (1 g/l).

B. Molecular analyses of transgenic plants:

Molecular analyses of T_0 putative transgenic wheat plants started by making PCR of the *bar* gene in the two putative transgenics with the rice *HAL2*-like (*RHL*) gene to test its presence in the wheat genomic background and results were positive (as shown in the last report).

As for the one T_0 putative transgenic wheat plant with *P5CS* gene for proline accumulation, PCR of the gene to test its presence in the wheat genomic background was positive (Figure 2). The development of normal seeds from this transgenic wheat plant is a challenge. After collecting the transgenic seeds, salinity experiment (100 mM NaCl) will be conducted sometimes in winter 2002 to test the expression of the gene in the transgenic under stress. However, more transgenic events ought to be accumulated to secure the expression of this gene and devoid the position effects against the wheat genomic background.

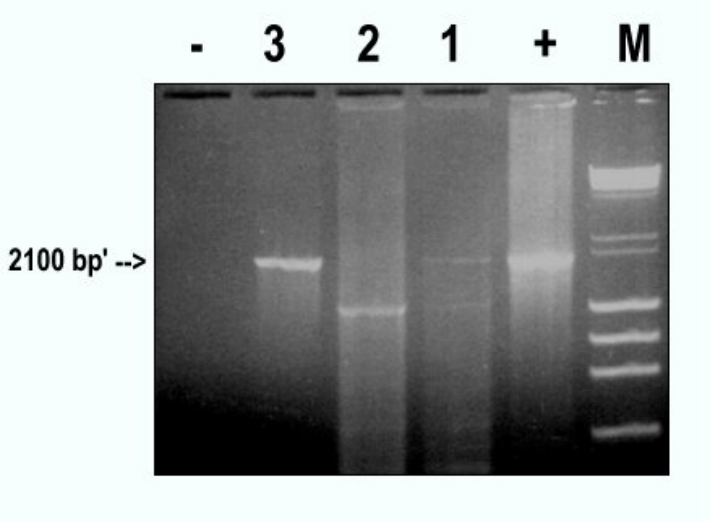

Figure 2. PCR products of *P5CS* gene (2100 bp) in one putative transgenic (3) with pNM plasmid, two non-transgenics, positive control of plasmid DNA (+), and negative control of W.T. plant (-). M refers to DNA standards of HaeIIIϕX/HindIIIλDNA.

C. Evaluation and performance of transgenics:

Salinity experiments (100 mM NaCl) have been conducted during winter 2001 to test the expression of the rice *HAL2*-like (*RHL*) gene in the two transgenics under stress. The overall results indicated the low performance of both transgenic plants under stress condition as indicated by the yield and its attributes (Table 1). Questions regarding the role of this gene in conferring tolerance against abiotic stress are raised. However, more transgenics ought to be accumulated to reach a final decision whether to utilize this gene for further plant transformation experiments to improve tolerance against abiotic stresses in wheat as well as other cereals or not.

Table 1. Performance of *HAL2*-like transgenic versus non-transgenic plants as yield and its attributes under normal as well as salt stress (100 mM NaCl) conditions.

	Genotype	Control	Treatment	Mean
Plant height				
	Transgenic	69.12ab	67.97b	68.54A
	Non-transgenic	74.63a	69.08ab	71.86A
	Mean	71.88A	68.53A	
Length of main spike				
	Transgenic	11.35b	10.48b	10.92A
	Non-transgenic	64.90a	11.10b	38.00A
	Mean	38.13A	10.79B	
Weight of main spike				
	Transgenic	2.55a	2.17a	2.36A
	Non-transgenic	2.63a	2.15a	2.39A
	Mean	2.59A	2.16B	
No. grains in main spike				
	Transgenic	51.17b	39.50c	45.33B
	Non-transgenic	68.60a	46.50bc	57.55A
	Mean	59.88A	43.00B	
Grain yield in main spike				
	Transgenic	1.82ab	1.48b	1.65A
	Non-transgenic	2.10a	1.57b	1.83A
	Mean	1.96A	1.52B	
Total grain yield				
	Transgenic	2.18a	1.49c	1.83B
	Non-transgenic	2.31a	1.84b	2.07A
	Mean	2.24A	1.66B	
Total biomass				
	Transgenic	5.52b	3.82c	4.67B
	Non-transgenic	7.18a	5.42b	6.30A
	Mean	6.35A	4.62B	

Highlights of significant achievements

Through this collaborative project, we expect to develop commercial transgenic wheat seeds at AGERI with improved tolerance to environmental stresses. T_2 and T_3 seeds will be developed and tested under environmental stress conditions before the commercialization process. Besides, the novel genes to be isolated at OSU will be patented and commercialized as soon as functions are proven.

The issue of the counterpart shares after the completion of this project has not been raised yet. But as for AGERI, there is a specialized unit as well as an IPR office that can go through the details of commercialization and distribution for both project counterparts.

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Tomato transformation for Development of Geminivirus Resistance

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Problem Definition

The whitefly *Bemisia tabaci* (Gennadius) is a widely distributed pest of many important food and fiber crops and it is also a vector of more than 70 plant-infecting viruses including geminiviruses. Tomato yellow leaf curl virus (TYLCV), a member of whitefly transmitted geminiviruses, is one of the most devastating viral diseases of cultivated tomato (*Lycopersicon esculentum*) in tropical and subtropical regions worldwide and losses of up to 100% are frequent. In many regions, TYLCV is the main limiting factor in tomato production. The causal agents are a group of geminivirus species belonging to the genus Begomovirus of the family Geminiviridae, all of them named Tomato yellow leaf curl virus (TYLCV). There has been almost 40 years of research on TYLCV epidemics and intensive research programs have been conducted to find solutions to the severe problem caused by these viruses. Geminiviruses cause devastating plant diseases to many crops in Egypt including tomato, clover, cotton, cucumbers, melon, faba bean, altheae. Intercropping (such as cucumber or squash) is used as trap crops to avoid virus transmission to the main cultivated crop.In Egypt, tomatoes are cultivated on 170,000 hectares, one-third the cultivated area devoted to vegetable crops. Tomatoes are considered an export crop, so it contributes significantly the Egyptian economy. In 2000, the total world production of tomatoes was estimated to be 100 million tons. Egypt produced about 6.2% of the total world production. In Egypt, tomatoes are infected by two different whitefly transmitted geminiviruses, tomato yellow leaf curl virus (TYLCV) and tomato yellow mosaic virus (TYMV).

The conventional breeding methods used to control viral diseases are poorly efficient or non-existent. On the other hand, there are no reliable means for controlling or reducing whitefly populations. Molecular genetics and plant transformation techniques provided new tools to introduce foreign genes into plant tissues without comprising other economic characters of the existing cultivar. Viral derived genes were used to engineer tomatoes for viral resistance.

The coat protein (CP) of geminiviruses is involved in a number of processes during the life cycle of the virus. The predominant function is encapsidation of single-stranded DNA and formation of the virus particle to protect viral DNA during transmission The whitefly endosymbiotic bacteria produces GroEL that interacts in the insect hemolymph with particles of tomato yellow leaf curl virus (TYLCV),

ensuring the safe circulative transmission of the virus. It was found that the GroEL-CP interaction in the hemolymph is a necessary condition for circulative transmission. This symbiotic protein attacked the attention of many scientists to use it for controlling the whiteflies.

General Objectives

1. To increase tomato yield, quality and horticultural value.
2. To reduce pesticide input.
3. To produce tomato cultivars resistant to whitefly transmitted geminiviruses.
4. To commercialize TYLCV resistant tomatoes with improved overall yield and high quality for export.

Technical Approaches:

1) Several approaches have been reported for the development of transgenic resistance against whitefly-transmitted geminiviruses. The approaches that are being followed for introducing TYLCV resistance at Egypt are:

   a) Expression of dominant mutation of the viral-replicase gene.

   b) Expression of antisense RNA against complementary sense gene.

   c) Over expression of coat protein in transgenic plant.

   d) Over expression of movement protein in transgenic plants.

   e) Expression of a virus induced cytotoxin gene in transgenic plants.

2) The virus insect relationship has been studied to find a way to control the insect vector of the virus.

3) Evaluation the efficiency of expression of viral promoter to be used as a plant promoter to control the expression of the introduced genes into plants.

Achievements

1) Strategies used to development transgenic tomatoes resistance against whitefly-transmitted geminiviruses

a) Expression of dominant mutation for the viral replicase (C_1) gene:

The viral replicase gene is a multifunctional protein whose activity comprises; recognition and binding to sequence motifs in left part of intergenic region, DNA cleavage to initiate regulation within the conserved nonanucleotide TAATATTAC, ATPase activity indispensable for viral DNA replication and repress it's own synthesis at the transcription level.

Dominant negative mutation can be obtained by introducing mutation in domains responsible

for the ATPase activity and the DNA cleavage sites. However, plants producing mutations in those sites did not expressed the mutated protein as it has the movement protein gene overlapped inside the replicase gene causing the expression of the Mp inside of the mutated Rep protein. Therefore, it was necessary to silent the expression of Mp but mutating its first ATG codon. Mutation have been obtained and will be used to produce transgenic tomato plants to study the effect of dominant mutation of the Rep protein on viral resistance.

b) Development of a recombinant DNA construct for resistance against TYLCV based on virus-induced expression of cytotoxin gene:

RNase is naturally expressed in any living organism and it's over expression causes a degradation of cytoplasmic RNA and death of the cell. The expression of RNase-barnase is derived by viral coat protein promoter and cloned in the plant binary vector pBin19. The promoter is activated in trans by one of the viral gene product (C2). The activation of transgene expression during virus infection avoids the constitutive expression of the cytotoxin gene. A set of primers were designed to amplify the viral Cp-promoter of TYLCV after addition of necessary restriction sites to ensure that the promoter is in frame with the barnase gene. The constructed plasmid (pSUS102) was used to transform local tomato cultivars and transgenic plants showed resistance to the virus.

Plants resulting from cultivating T1 seeds in greenhouse biocontainment were challenged with viruliferous whiteflies together with a proper source of infection. Out of 83 transgenic plants, 62 showed no symptoms giving rise to a ratio of 3:1 as expected in the T2. DNA was extracted from T2 transgenic tomato plants and evaluated using PCR/DNA probe to detect the introduced construct in the healthy plants. Seeds were collected from T2 transgenic tomato plants which showed resistance to whitefly transmitted geminivirus. The collected seeds were cultivated in a commercial greenhouse (as the local tomato cultivar, GH75, is a greenhouse cultivar) with source of infection. About 180 transgenic seeds from 25 lines as well as non-transgenic seeds (control) were cultivated in commercial greenhouse. The seeds were arranged in a random manner of the different lines and the control. Plants were subjected to natural infestation with the viruliferous whiteflies. Four weeks later after transplantation, geminiviral symptoms were weekly recorded. After three months of transplantation, 57 plants out of 180 (31.6%) showed typical TYLCV symptoms (Fig.1). Seeds collected from healthy tomato plants will be used for crossing with the other parents after identifying the homologous plants for the introduced construct. The hybrid seeds will be ready for commercialization as they will contain the desired characters for that purpose.

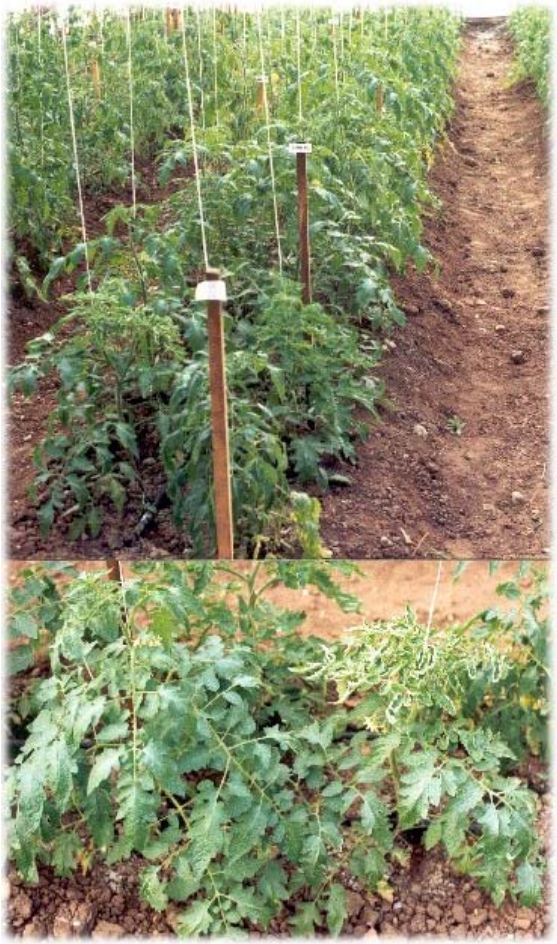

Figure 1: T_2 plants cultivated, carrying the virus-induced expression of cytotoxin gene strategy, in the greenhouse biocontainment challenged with viruliferous whiteflies.

2) The virus insect relationship has been studied to find a way to control the insect vector of the virus.

The acquisition access period required for transmission of TYLCV by *B. tabaci* was studied after feeding the insects on cotton plants for 45 day to obtain virus free whiteflies then fed on infected tomato plants with TYLCV to acquire the virus and insect samples were collected periodically at Zero time, 30 min, 1 hr, 2 hr to 5 hr then 1 and 2 overnights. PCR reaction, as a fast and reliable tool, was used to detect the viral genome after the different acquisition period using the viral coat protein specific primers. The expected 770 bp fragment was detected in DNA extracted from insects after 4 hrs feeding on infected tomato. It is well known that acquisition of a virus depends on the virus titer in the infected plant, the ability of insect to ingest the virus and the passage of the virus through the midgut wall and subsequent survival in the insect vector.

For further understanding the whitefly-geminivirus complex, the ability of viruliferous whiteflies to inoculate tomato plants was determined. Viruliferous insects were spread on ten healthy tomato plants at the age of two to three true leaves and kept for 48 hr in an insect wooden proof cage in the

greenhouse. Plants were monitored for the appearance of the virus symptoms after different period of time post inoculation. The correlation between development of disease symptoms and accumulation of TYLCV-DNA has been investigated. TYLCV-DNA can be detected in purified DNA from infected tissues by PCR analysis with TYLCV-specific primers. This method showed that the time course of TYLCV-DNA accumulation in tissues was clear 9 days post inoculation. While disease symptoms became visible 15 days post-inoculation for only 30% of the inoculated plants and 100% visible 30 days post-inoculation. These results indicated that TYLCV-DNA was detectable in plant tissues at least 6 days before disease symptoms were observed. The lag between appearance of detectable amount of viral DNA and that of disease symptoms could be used for early detection of TYLCV infection in the field and greenhouse.

To study the viral coat protein function in the viral circulation within the insect hemolymph, gene encoding the TYLCV coat protein was fused with the GFP gene to be expressed as one protein. The autofluorescent protein, GFP, can be visualized non-obtrusively in living cells with a standard fluorescent; its role is to transduce, by energy transfer, the blue chemiluminescence of another protein, aequorin, into green fluorescent light. It can be used in transient expression, trafficking and protein localization studies, protein purification and transgenic plants and animals. The fused protein was expressed in E. coli cells and used to visualize the Cp accumulation within the whitefly by membrane feeding on sucrose solution containing the fused protein. It was observed that the Cp by it self (not necessary to be virus particles) can penetrate the insect gut and circulate in it's hemolymph. It was also observed that after 24 hrs feeding, the Cp accumulated in the abdomen, specially in the ovary emitting a green fluorescent light under the fluorescent microscope as shown in figure (2), but no emission of green light was observed in the thorax. However, after 48 hrs feeding the Cp could be seen in both thorax and abdomen of the insect and even in the hemolymph of the wings and legs. The sucker also emitted green light after feeding as shown in figure (3).

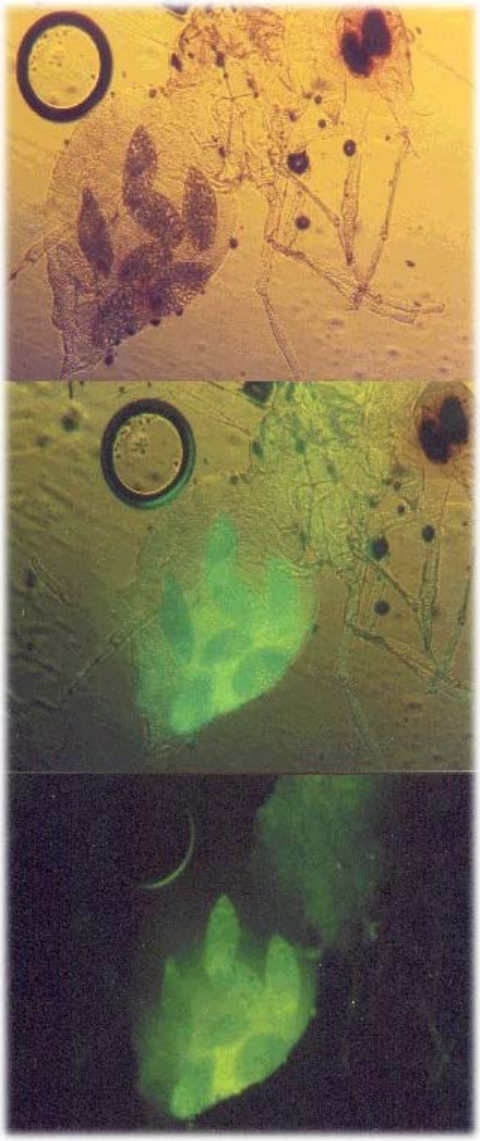

Figure (2): Fluorescent microscope examination of whitefly *B. tabaci* after feeding for 24 hr on the fused protein Cp::GFP **(a)** Insects under light, **(b)** under both light & UV and **(c)** under UV only, showing the emission of green fluorescent light where Cp::GFP is presented.

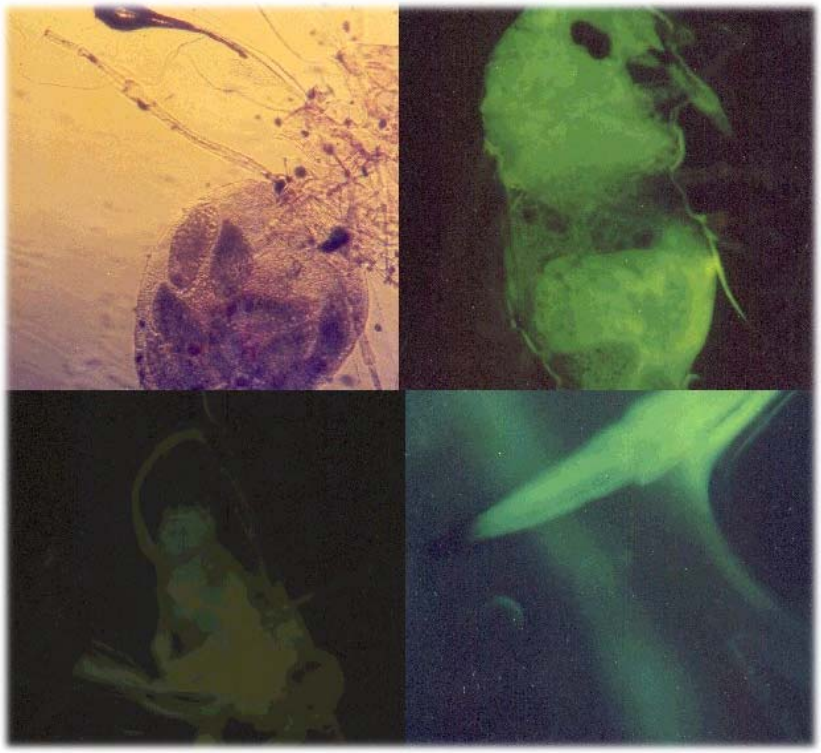

Figure (3): Fluorescent microscope examination of whitefly *B. tabaci* after feeding on the fused protein Cp::GFP for 48 hr. **(a)** Insects under light and **(b)** under UV, showing the expression of green fluorescent light in all the body. **(c)** Whitefly fed on sucrose solution without the fused protein used as negative control**. (d)** Insect sucker after feeding on the fused protein showing green emission where the fused protein is presented.

3) Evaluation the efficiency of expression of viral promoter to be used as a plant promoter to control the expression of the introduced genes into plants.

The plant promoter is the key for gene expression in plant tissues. Almost all identified promoters are patent and a permit is needed for commercialization use. Therefore, the viral promoters were evaluated for that purpose. First the replicase promoter was evaluated using the gus reporter gene and unfortunately it showed a low expression whether in the host plants or other dicotyledonous plants. The Cp promoter is a trans activated promoter, meaning it does not show any expression unless the viral C2 protein is expressed in the cells. The viral movement protein promoter was also evaluated by cloning it upstream of the gus reporter gene and evaluated and compared with the 35S promoter in tomato and squash tissues (Fig. 4). The expression of the both promoter did not show a significant differences between the (Fig. 5), indicating that the cloned promoter is capable for using in plant transformation using gene of interest in dicotyledonous plants. Also, the promoter will be evaluated for its expression in monocotyledonous plants.

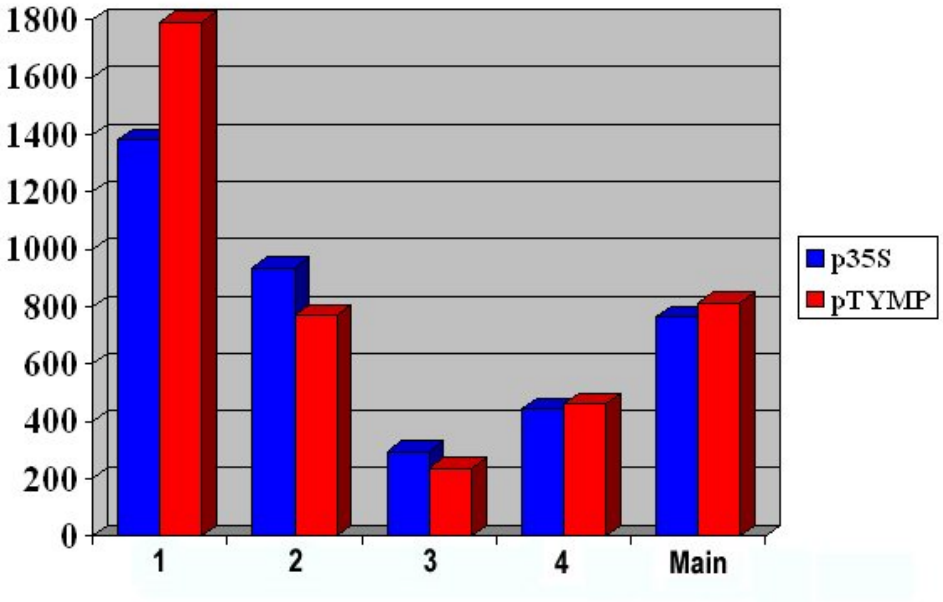

Figure (4): Evaluation of the Mp promoter efficiency in expressing the *gus* gene compared to the 35S promoter. Four different experiments (1-4) have been performed using different conditions to compare between the expressions of the two promoters.

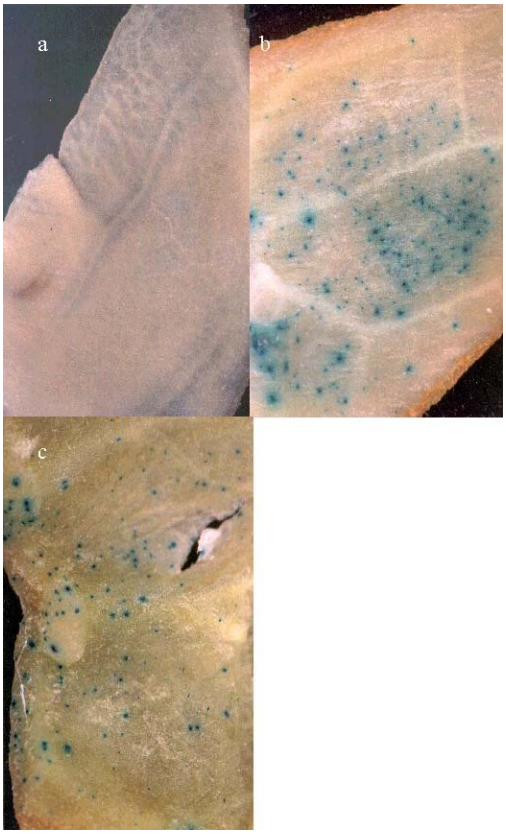

Figure (5): Histochemical staining of transformed plant with (b) Mp-promoter and (b) 35S promoter using the ballistic DNA gun, compared to the non-transformed.

Molecular Characterization of Insect Midgut Toxin Receptors for Circumventing Resistance to Toxins of *Bacillus thuringiensis*

Lead Principal Investigator:

Lee A. Bulla, Jr., University of Dallas at Texas

Project Partners

Magdy Madkour, AGERI, Egypt

Overall Project Goal

The overall objective of the research project is to determine the molecular mechanism(s) of insect resistance to the insecticidal toxins (Cry toxins) of *Bacillus thuringiensis*.

Importance of the Problem/Justification for Project

There are approximately 25 subspecies of *Bacillus thuringiensis* (Bt) that produce an array of parasporal crystalline proteins, known as Cry toxins, which are lethal to a variety of agriculturally important insects. Because the Cry proteins of the various subspecies have quantifiable specific toxicity to a wide range of different insects and because they pose no demonstrable threat to other nontarget organisms or to the environment, there is increasing interest in the use of *B. thuringiensis* as a biopesticide. Certainly, the use of Bt has intensified and, during the past several years, *cry* genes have been used to transform plants to render them insect resistant. Transgenic plants carrying the toxin genes of Bt have been introduced into the United States and efforts are underway to utilize such plants in Egypt and the Middle East. Several Bt biopesticides have been marketed and used in Egypt and the Middle East for crop protection. Widespread use of Bt-based bioinsecticides may be causing insects to become resistant to certain Cry toxins. The rise in and expansion of insect infestations due to global warming also is disconcerting and, therefore, it is readily apparent that attention should be paid to improving transgenic plants carrying *cry* genes as well as those biopesticides formulated with Bt. Designing new and novel Cry toxin molecules or synthetic substitutes derived there from should help circumvent the potentially serious problem of insect resistance to Bt.

Project Background

The cotton leafworm (*Spodoptera littoralis*) is a major problem in Egypt because it destroys horticultural crops such as tomatoes, potatoes and cucurbits as well as corn. Bt insecticidal toxins effectively control the leafworm. Recently, however, the insect has exhibited some resistance to Bt toxins. Therefore, it is important to gain a better understanding of the molecular properties that

mediate toxicity to insects such as the cotton leafworm. Several Bt toxin-binding proteins have been identified in various insects. However, only one receptor molecule, BT-R_1 from the tobacco hornworm (*Manduca sexta*), has been cloned, sequenced and determined to mediate insect toxicity. Several homologues of BT-R_1 have also been identified by the principal investigators in insect pests important to Egyptian and American agriculture. The toxin-binding site of BT-R_1 has been determined by collaborative efforts between the co-principal investigators, and, its physical structure is under investigation. Knowing the mechanism of binding will allow us to ascertain the parameters that dictate normal binding versus abnormal or reduced binding in resistant insect species. However, Cry toxin-receptor binding is not the only parameter that contributes to resistance to the toxin. Therefore, research is underway to determine all factors involved in the resistance phenomenon. The resistance system that is being pursued currently is the Colorado potato beetle in conjunction with Dr. Leah Bauer at Michigan State University. We do not have access to any resistant strains of the cotton leafworm, but, when they become available, we will compare the potato beetle system to that of the leafworm.

Rationale for Approach

There is little knowledge about the mechanism(s) of resistance development against Bt toxins in insects. One possible mechanism includes decrease in the binding of toxin to insect midgut in some insects. However, reduced binding is not always correlated with resistance to Bt suggesting that other mechanisms are involved in the resistance development. There also is supporting evidence that proteases in the insect gut may take part in the evolution of resistance to Bt toxin. For instance, proteases from a strain of *Heliothis virescens* resistant to *B. thuringiensis* subsp. *kurstaki* HD-73 have been reported to process the Cry1Ac protoxin more slowly and to degrade toxin faster than enzymes from a susceptible strain. There is circumstantial evidence for an increase in the specific activity of gut proteases in later developmental stages of larvae than in earlier ones. This activity has been associated with a loss of sensitivity to Cry1C, possibly, due to an increase in the degradation of toxin. Two resistant strains of the Indian meal moth, *Plodia interpunctella*, have been found to lack a major gut protease that is involved in protoxin activation. These studies indicate that changes in the activity and/or composition of gut proteases may be involved in decreased susceptibility to Cry toxin in insects.

Previous Research

Colorado potato beetle studies. The protease activity profiles and toxin-binding capacities in the midgut of a strain of Colorado potato beetle (CPB) that has developed resistance to the Cry3Aa toxin of *B. thuringiensis* subsp. t*enebrionis* were investigated. Histological examination revealed that the structural integrity of the midgut tissue in the toxin-resistant (R) insect was retained whereas the same tissue was devastated by toxin action in the susceptible (S) strain. Function-based activity profiling using zymographic gels showed specific proteolytic bands present in midgut extracts and brush border membrane vesicles (BBMV) of the R strain not apparent in the S strain. Aminopeptidase activity associated with insect midgut was higher in the R strain than in the S strain. Enzymatic processing of toxin did not differ in either strain and, apparently, is not a factor in resistance. BBMV from the R strain bound ~60% less toxin than BBMV from the S strain whereas the kinetics of toxin saturation of BBMV was 30 times less in the R strain than in the S strain.

However, homologous competition inhibition binding of ^{125}I-Cry3Aa to BBMV did not reveal any differences in binding affinity (K_d H 0.1 μM) between the S and R strains. The results indicate that resistance by the CPB to the Cry3Aa toxin correlates with specific alterations in protease activity in the midgut as well as with decreased toxin binding. These features reflect adaptive responses that render the insect refractory to toxin action, making this insect an ideal model to study host innate responses and adaptive changes brought on by bacterial toxin interaction.

Cry toxin binding region studies of BT-R1 isolated from *M. sexta*. Many subspecies of the soil bacterium *Bacillus thuringiensis* produce various parasporal crystal proteins, also known as Cry toxins, which exhibit insecticidal activity upon binding to specific receptors in the midgut of susceptible insects. One such receptor, BT-R_1 (210 kDa), is a cadherin located in the midgut epithelium of the tobacco hornworm, *Manduca sexta*. It has a high binding affinity (K_d ~ 1nM) for the Cry1A toxins of *B. thuringiensis.* Truncation analysis of BT-R_1 revealed that the only fragment capable of binding the Cry1A toxins of *B. thuringiensis* was a contiguous 169-amino acid sequence adjacent to the membrane-proximal extracellular domain. The purified toxin-binding fragment acted as an antagonist to Cry1Ab toxin by blocking the binding of toxin to the tobacco hornworm midgut and inhibiting insecticidal action. Exogenous Cry1Ab toxin bound to intact COS-7 cells expressing BT-R_1 cDNA, subsequently killing the cells. Recruitment of BT-R_1 by *B. thuringiensis* indicates that the bacterium interacts with a specific cell adhesion molecule during its pathogenesis. Apparently, Cry toxins, like other bacterial toxins, attack epithelial barriers by targeting cell adhesion molecules within susceptible insect hosts.

Targeting host cadherins and other cell junction molecules may be representative of those bacteria that disrupt or evade epithelial barriers in their hosts. Evidently, the pathogenicity of *B. thuringiensis* shares a common mechanism(s) with other bacterial pathogens that target host tissues. Many of the open reading frames (ORFs) in the *Bacillus anthracis* virulence plasmid pXO1 show 80 and 98% similarity to genes in isolates of closely related *Bacillus* species, including *B. cereus* and *B. thuringiensis*. However, it remains to be determined whether there are commonalities among *B. thuringiensis*, *B. anthracis* and *B. cereus*, which have a high degree of genetic relatedness and whose toxins target cell-surface receptors). Nevertheless, recruitment of BT-R_1 by *B. thuringiensis* to overpower a susceptible host is a model system to study bacterial pathogenesis and to examine co-evolutionary relationships among insects and entomopathogens.

Specific Project Objectives

1. Investigate the effects of metal ions and chelating agents on the integrity of BT-R_1 and Cry1Ab toxin binding to the molecule
2. Determine how calcium ions in particular influence the structure of the BT-R_1 ectodomain
3. Determine how BT-R_1 is cleaved specifically in the presence and absence of EDTA
4. Determine whether BT-R_1 becomes refractory to EDTA-induced cleavage after purification by a variety of procedures
5. Establish a model for calcium-dependent cleavage of BT-R_1.

Research Progress

Studies on the proteolysis of BT-R_1, a developmentally important cadherin receptor from *M. sexta*.

Functional characteristics of cadherin have been attributed to their Ca^{++}-dependent structural features and molecular interactions in cell adhesion events. We investigated the influence of calcium on the structural integrity of gut-specific epithelial cadherin, BT-R_1 (M_r = 210 kDa), in brush border membrane vesicles (BBMV) from the tobacco hornworm *Manduca sexta*. BT-R_1 is expressed during larval growth and development of *M. sexta*. It also is targeted by Cry1A toxins of the entomopathogenic bacterium *Bacillus thuringiensis*. Circular dichroism studies revealed the effect of calcium on the secondary structure of the BT-R_1 ectodomain. BT-R_1 stays intact on BBMV, but, chelation of cations with EDTA permitted proteolytic cleavage of the ectodomain. Cleavage was not prevented by specific inhibitors of metalloproteases nor by various inhibitors for serine, aspartic and cysteine proteases. Binding of Cry1Ab toxin to BBMV also did not block the cleavage of BT-R_1 in the presence of EDTA. When BBMV was purified by gel filtration, EDTA did not promote the cleavage of BT-R_1. The results indicate that there is a specific protease(s) that modulates molecular interactions of the BT-R_1 ectodomain, which is dependent on calcium ions. Specific cleavage of BT-R_1 may be implicated in cell adhesion during growth and development of *M. sexta* gut epithelium.

Structural model for BT-R_1. BT-R_1 is a target receptor for the Cry1A toxins produced by the soil bacterium *Bacillus thuringiensis* and has evolutionary significance to the entomopathogenicity of the bacterium. The predicted structure and domain architecture of BT-R_1 (Fig.1), based on the deduced amino acid sequence and domain organization of cadherins, together with the specific expression pattern in *M. sexta* larvae strongly suggest that BT-R_1 functions as a heterophilic cell adhesion molecule in the midgut during larval growth and development.

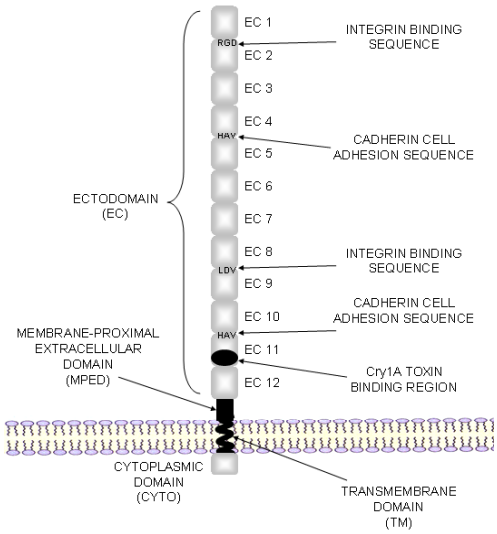

Fig 1. Model of BT-R_1 structure. Proposed structure of the cadherin receptor BT-R_1 is based on the domain organization of the cadherin superfamily of proteins. BT-R_1 is composed of four domains: (i) ectodomain (EC), (ii) membrane-proximal extracellular domain (MPED), (iii)

transmembrane domain and (iv) cytoplasmic domain (CYTO). Downstream of a putative membrane signal sequence, the ectodomain consists of 12 cadherin repeats made of β-sheets that are structured as ectodomain modules (EC1 through EC12). The ectodomain harbors a pair of cell-adhesion sequences, HAV (His-Ala-Val) that is characteristic to all cadherins and two integrin binding sequences RGD (Arg-Gly-Asp) and LDV (Leu-Asp-Val). The Cry1A toxin-binding region folds into the EC11, close to MPED.

Effects of Ca^{2+} on the conformation of BT-R_1. To determine whether calcium influenced the conformation of BT-R_1, the effects of Ca^{2+} ions on the CD spectrum of a segment encompassing EC10 and EC12 of the BT-R_1 ectodomain was examined. The spectrum of BT-R_1 fragment (Fig. 2) was similar to the spectrum of E-cadherin ectodomain, which has β-sheet structures as the main secondary structural element. The shape of the spectrum for the BT-R_1 fragment (Fig. 2) indicates the presence of a large fraction of β-structure in the molecule. The β-structure conformation was present both in the absence and in the presence of Ca^{++}. However, structural changes were observed upon binding of Ca^{2+}, which caused a significant decrease in the molar elipticity at 215nm (Fig. 2) and suggests that BT-R_1 ectodomain undergoes structural changes upon binding of Ca^{2+} to the molecule. The observed difference between the spectra is above the error levels. Furthermore, the nature and the magnitude of the decrease were similar to that described for other cadherins. Thus, Ca^{2+}-dependent structural transformation of the BT-R_1 ecdomain reflects a change in the conformation of the fragment.

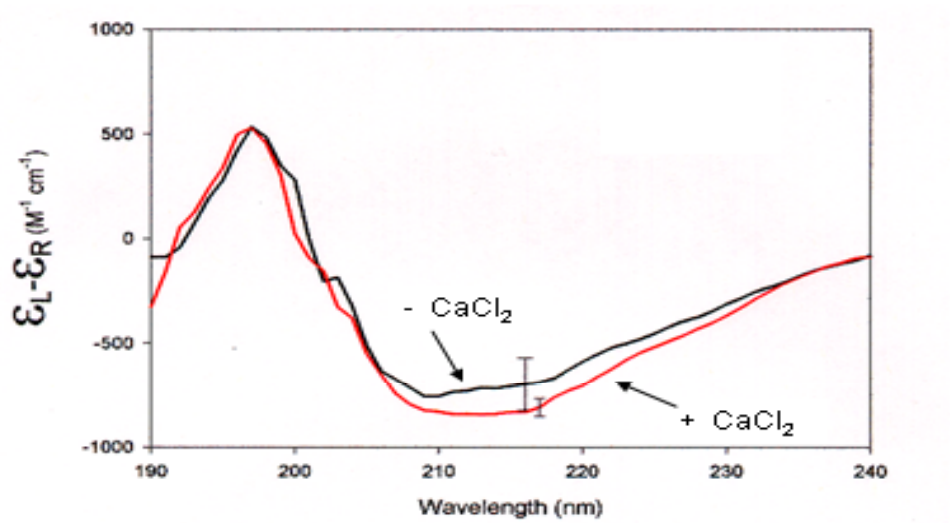

Fig. 2. CD spectra of BT-R1 ectodomain fragment in the presence and absence of Ca2+.

Specificity and affinity of Cry1A toxin binding to brush border membrane vesicles

(BBMV). The Cry1A toxins of *B. thuringiensis* bind specifically and with high-affinity to BT-R_1. ^{125}I-labelled Cry 1Ab toxin was used in Western blot experiments for specific detection of BT-R_1 bands in proteins present on BBMV obtained from *M. sexta* larval guts. The effects of different metal ions and chelating agents on binding of ^{125}I-Cry1Ab to BT-R_1 were tested by ligand blot analysis (Fig. 3). The presence of 10 mM $CaCl_2$ or $SrCl_2$ or $MgCl_2$ in the incubation mixture did not change the binding of ^{125}I-Cry1Ab to 210 kDa BT-R_1 fragment (Fig 3, lanes 1-3). However, binding of the toxin to BT-R_1 was reduced markedly whereas binding to 145- and 120-kDa fragments was observed in the presence of EDTA (Fig. 3, lane 4).

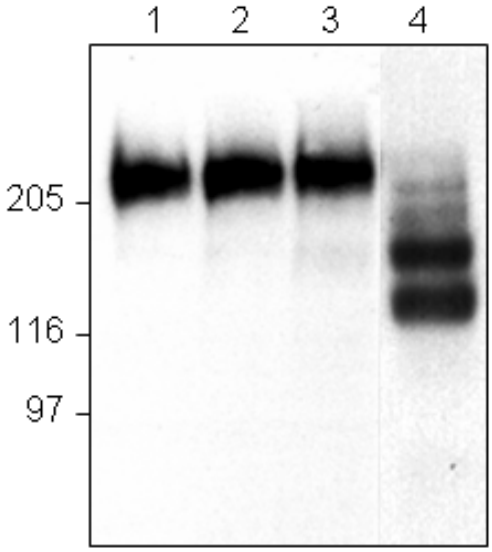

Fig. 3. Ligand blot analysis of BT-R_1 and its cleavage fragments in the presence of metal ions and EDTA.

Time-course ligand blot analysis of Cry toxin binding to the BT-R_1 cleavage fragments. Time-course ligand blot analysis showed that increased binding of the radiolabeled toxin to the 145- and 120-kDa fragments accompany a corresponding decrease in binding of the toxin to the 210 kDa BT-R_1 (Fig. 4A). Total amount of ^{125}I-Cry1Ab bound to BT-R_1 and to the 145- and 120-kDa fragments was approximately the same before and after incubation with EDTA (Fig. 4B). The results suggest that incubation of BBMV did not expose some other proteins that were not accessible on the membrane, and, that the 145- and 120-kDa fragments are indeed formed by sequential cleavage of BT-R_1. Treatment of BBMV with 10 mM EDTA for 24 hr resulted in the disappearance of ~85% of the 210-kDa BT-R_1 band and led to the accumulation of 145- and 120-kDa fragments (Fig. 4A, lane 4 and Fig. 4B). After 4 hr and 8 hr of EDTA treatment of BBMV, addition of Ca^{2+} to the incubation mixture blocked the accumulation of the 145- and 120-kDa fragments (Fig. 4C), and, further incubation of BBMV with EDTA and $CaCl_2$ up to 24 hr did not result in accumulation of the 145- and 120-kDa fragments (Fig. 4C and 4D). Furthermore, the 145- and 120-kDa fragments were not produced at pH 7 and below, because EDTA is not an effective chelating agent in pH 7 and lower (Fig. 5). Similar results also were obtained by using EGTA although the rate of formation of the 145- and 120-kDa fragments was higher in the presence of EDTA (data not shown).

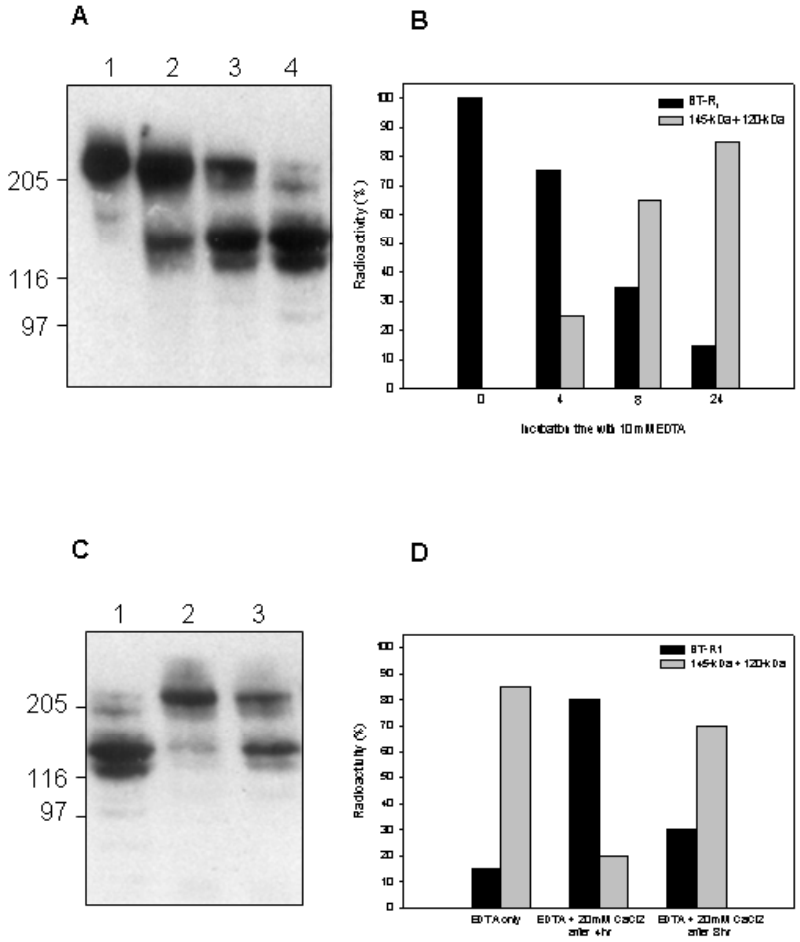

Fig. 4. Time-course ligand blot analysis of BT-R_1 and its cleavage fragments in the presence of EDTA and $CaCl_2$.

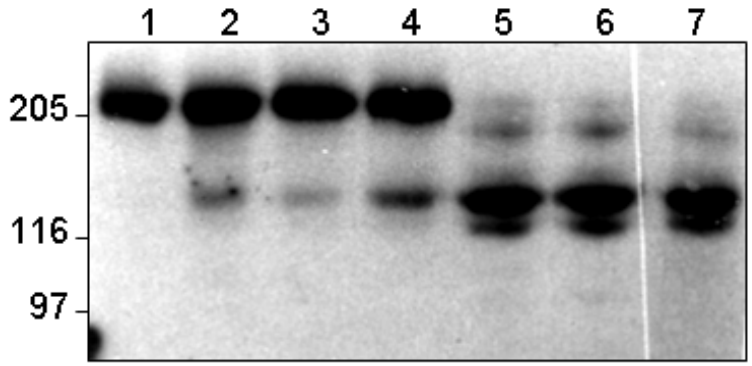

Fig. 5. Effect of pH on the EDTA-induced cleavage of BT-R_1 on the *M. sexta* BBMV.

Effect of protein reduction on EDTA-induced cleavage of BT-R_1. To test whether calcium-dependent cleavage of BT-R_1 is related to a reduced state of the protein, BBMV were treated with 1 mM DTT (1 mM) added to the incubation mixture. As can be seen in Fig. 6,protein reduced with DTT did not prevent EDTA-induced cleavage. The cleavage is not due to a proteolytic activity associated with BT-R_1 itself, because, partial purification of BT-R_1 by gel filtration excludes proteolytic activity and incubation of gel filtration purified BT-R_1 with EDTA did not result in formation of 145- and 120-kDa fragments (Fig.7). Thus, there is a specific protease(s) that resides on the BBMV that appears to be responsible for specific proteolytic modification of BT-R_1.

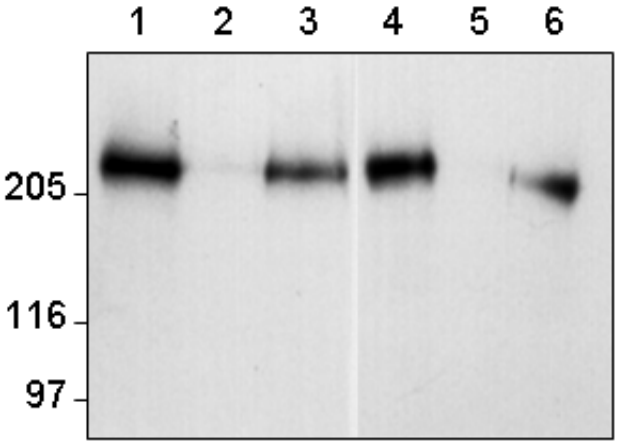

Fig. 6. Effect of DTT treatment on EDTA-induced cleavage of BT-R_1 in BBMV.

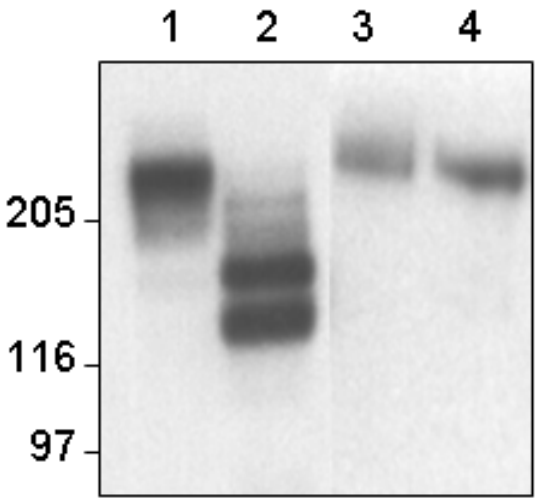

Fig. 7. Effect of gel chromatography on EDTA-induced cleavage of BT-R_1 in BBMV.

Effect of EDTA on BBMV proteins. No general degradation of BBMV proteins occurred upon treatment with EDTA. Coomassie blue stained SDS-PAGE gel of BBMV proteins before and after addition of EDTA showed that the profile of the majority of protein bands remain unchanged, except for the protein at around ~210-kDa, which noticeably disappeared after incubation with EDTA (Fig. 8). These results indicate that chelating divalent metal ions increases the susceptibility of BT-R_1 to specific cleavage, either by exposing a segment of the protein, which is normally resistant to proteolysis, or, removal of the metal ions activates a specific protease on BBMV. The results also suggest that, similar to other membrane protein ectodomains, including cadherins, the BT-R_1 ectodomain is subjected to specific proteolytic cleavage.

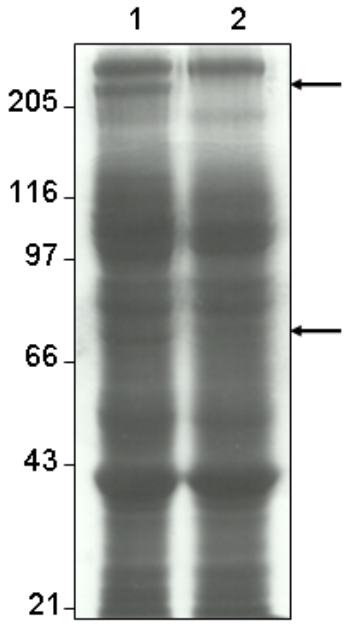

Fig. 8. Coomassie blue stained SDS gel of *M. sexta* BBMV proteins before (lane 1) and after (lane 2) EDTA treatment.

Effect of protease inhibitors on BT-R_1 cleavage. To understand the nature of the proteolytic activity responsible for the cleavage of BT-R_1, different protease inhibitors were added to reaction mixtures containing BBMV and EDTA. The inhibitors had no effect, individually or in combination, on the cleavage of BT-R_1. In all cases, EDTA treatment resulted in the formation of 145- and 120-kDa fragments and the level of ^{125}I-Cry1Ab binding to the fragments were essentially unaffected (data not shown). Treatment of BBMV with chemically modifying reagents also did not affect the cleavage of BT-R_1 (data not shown).

Effect of Cry toxin binding to BBMV on BT-R_1 cleavage. We tested whether Cry1Ab binding to BBMV affected EDTA-induced formation of the 145-and 120-kDa fragments. Incubation of BBMV with unlabeled Cry1Ab toxin did not cause formation of specific cleavage fragments in the presence of Ca^{++} (Fig. 9). Toxin binding also did not inhibit the formation of the 145-and 120-kDa fragments in the presence of EDTA (Fig. 9) There was no noticeable reduction in ^{125}I-Cry1Ab toxin binding to the blots in these experiments. The results indicate that Cry toxin binding to BT-R_1 does not interfere with the specific cleavage sites on the receptor.

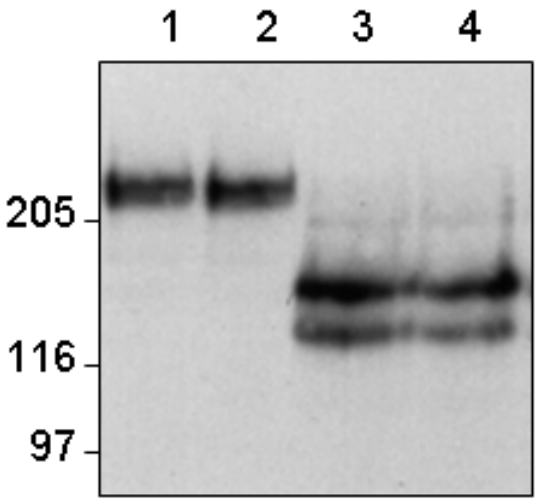

Fig. 9. Effect of Cry1Ab toxin binding to BBMV on EDTA-induced cleavage of BT-R_1.

To understand the pattern of EDTA-induced cleavage of BT-R_1, ^{125}I-Cry1Ab toxin binding to the soluble and membrane-bound fractions after EDTA treatment of BBMV for 24 hr was examined. Membrane bound and soluble proteins were separated by centrifugation and blotted on PVDF membrane. Radiolabeled toxin bound to the 145- and 120-kDa protein fragments only from the precipitated fraction (Fig. 10). The results indicate that a large portion of the BT-R_1 ectodomain containing the toxin-binding region remains attached to the membrane after cleavage of the molecule.

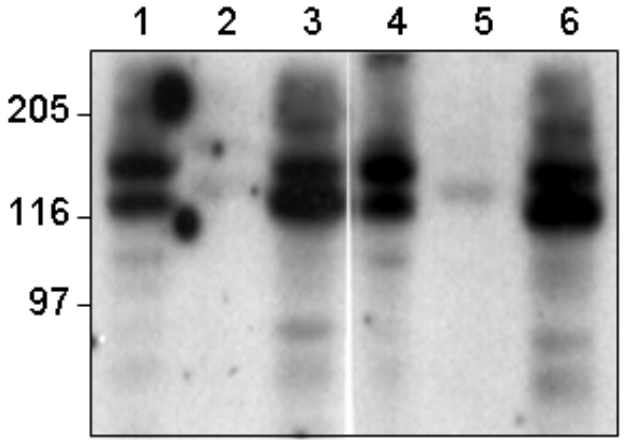

Fig. 10. Ligand blot analysis of soluble and membrane-bound fractions of EDTA-treated *M. sexta* BBMV proteins.

Discussion/Implications

EDTA-induced cleavage of BT-R_1 involves at least two cleavage sites, which are utilized sequentially (see model in Fig 11). According to this model, cleavage of BT-R_1 correlates with calcium removal and takes place at a region between EC3 and EC6, where BT-R_1 contains one of the potential cadherin cell adhesion recognition sequences (HAV) and displays an E-cadherin folding motif. Sensitivity to Ca^{+2} concentrations and structural changes related to removal of calcium might expose this region to proteases, resulting in the specific cleavage of BT-R_1. Furthermore, cleavage of BT-R_1 in this region may have functional significance such that, the molecule may be detached from the N-terminal portion containing the integrin binding sequence, RGD, as well as one of the potential cadherin binding sequences (HAV), while retaining the potential integrin binding sequence, LDV, and the other cadherin binding sequence, HAV. Thus, the scheme depicted in Fig. 11 could explain how specific cleavage modulates the adhesive properties of BT-R_1 depending on calcium ion concentration. In epithelial cell cultures, calcium removal abolishes homodimerization of cadherins while triggering formation of intercadherin interactions involving desmogleins and desmocollins. Such interactions are believed to function in the assembly of cell junctions and in their disassembly when calcium concentrations change. In fact, adhesion and contact-dependent interactions of cells with neighboring cells and with the extracellular matrix provide not only mechanical support but also necessary signals for the regulation of cell fate and morphology where novel cadherin interactions most likely maintain normal epithelial organization and differentiation of proliferating cells in epithelial tissues during growth and development.

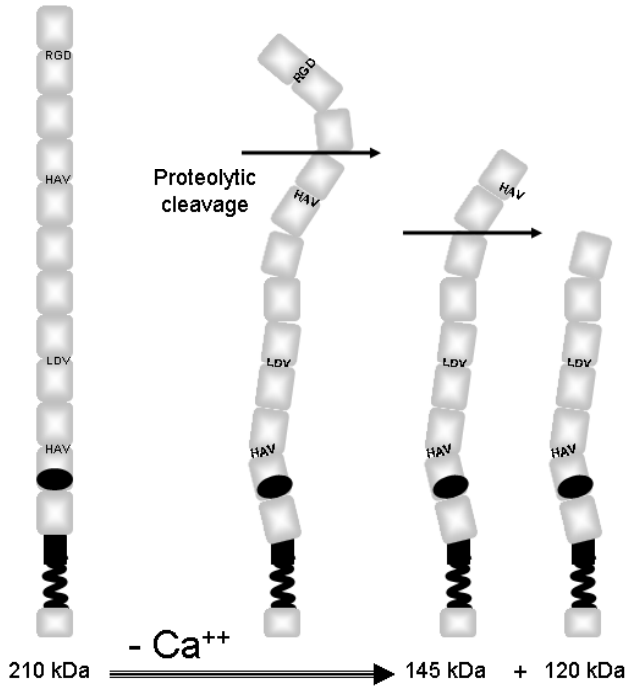

Fig. 11. Model of calcium-dependent cleavage the cadherin receptor BT-R_1.

Highlights of Significant Achievements

Many functionally diverse proteins such as cell-cell and cell-matrix adhesion molecules, cytokines, growth factors, and their receptors, are released from their membrane-anchored moieties by proteolysis; however, the proteinases mediating specific cleavage of these proteins remain mostly undefined. Despite the diversity in structure, cell type distribution, membrane topology, and cleavage site sequence of these shed ectodomains, there are evidences suggesting a common mechanism involving metalloproteases in ectodomain processing. One line of such evidence shows that the shedding of the extracellular domain of a number of cell surface proteins is blocked by metalloprotease inhibitors. Another line of the evidence comes from mutant cell lines that are defective in the release of multiple unrelated ectodomains. Metalloproteases (MMPs) are members of a family of at least 15 Zn^{++} dependent endopeptidases that function extracellularly. Membrane bound metalloproteases are very tightly complexed with their catalytic Zn atom and usually are unaffected by addition of EDTA.

Initial analysis showed that the proteolytic activity associated with the cleavage of BT-R_1 is not prevented by the metalloprotease inhibitors phosphoramidon, 1,10-phenanthroline, KD-IX-73-4 (inhibitor for selectin cleavage) and EDTA itself. Inhibitors of serine, thiol or aspartic proteases also did not affect calcium-dependent cleavage of BT-R_1 under a variety of experimental conditions. Possibly, the protease associated with cleavage of BT-R_1 may have a mode of action different from those established families of proteases because of the high pH (10.5) of the *M. sexta* midgut. Under such conditions lysine or tyrosine groups may be found in the active site of the protease because these side chains generally have pK values of ~10 and they may be better able to function in acid/base catalysis than the imidazole group of histidine, the thiol group of cysteine or the carboxylate groups of aspartate and glutamate. Addition of sulfo-NHS-acetate, however, which modifies the ε-amino groups of lysine residues, has no effect at a concentration of 5 mg/ml. At a concentration of 10 mg/ml proteolysis increased. Addition of NBSF, a tyrosine modifying reagent, also has no effect.

The proteolytic activity related to calcium dependent cleavage of BT-R_1 does not reside in BT-R_1 itself because separation of BT-R_1 from the proteolytic activity occurs when BBMV are solubilized in 0.5% CHAPS and passed through a gel filtration column (Fig.7). The BBMV protease appears to exhibit specificity for the cleavage of BT-R_1. This type of behavior is characteristic of membrane proteins, secretase and sheddase, of which many examples have now been reported. These proteases are responsible for the release or shedding of membrane anchored proteins in soluble forms including the extracellular segments of cadherins. The enzymes are mainly metalloproteases there is some evidence of involvement of serine proteases in certain cases. Certainly, isolation and characterization of the proteolytic activity related to the cleavage of BT-R_1 is an important aspect for future investigation.

Specific proteolytic cleavage and ectodomain shedding of membrane proteins are important aspects of cell regulation and cell interaction. Indeed, developmental and tissue-specific expression of the potentially heterophilic cadherin BT-R_1 in the gut of *M. sexta* larva supports the notion that BT-R_1 and the cleavage of its ectodomain may be critical in specific cell interactions in the gut epithelium. During the period of dynamic growth and cell proliferation and developmental tissue organization, the larval gut is remodeled while the epithelial tissue retains its structural and

functional integrity. Apparently, cell adhesion events are critical during developmental structuring of the tissues. Many of these functions most likely involve cell-cell adhesion events mediated by cadherins as well as activation of specific intracellular signaling pathways. However, further studies are necessary to elucidate the functional significance of Ca^{2+} dependent proteolysis of BT-R_1 in the larval gut epithelium. Determining the specific cleavage pattern, modulation of interactions and the clustering of cadherins on cell surfaces may provide insight into how specific cell adhesion events participate in epithelial cell proliferation, differentiation, cell migration, wound repair, programmed cell death and not to mention a variety of pathophysiological conditions.

Publications

Midboe, E.G., M. Candas, J.A. Dorsch and L.A. Bulla, Jr. 2001. Susceptibility of *Manduca sexta* Lrva to the Cry1Ab Toxin of *Bacillus thuringiensis* Correlates Inversely With the Developmental Expression of the Toxin Receptor BT-R_1. *SAAS Bulletin: Biochem. Biotechnol*. 14: 73-80.

Loseva O, M. Ibrahim, M. Candas, C.N. Koller, L.S. Bauer and LA Bulla, Jr. 2002. Changes in Protease Activity and Cry3Aa Toxin Binding in the Colorado Potato Beetle: Implications for Insect Resistance to *Bacillus thuringiensis* Toxins. *Insect Biochemistry and Molecular Biology.* 32:567-577.

John A. Dorsch, Mehmet Candas, Natalya B. Griko, Walid S. A. Maaty, Eric G. Midboe, Ratna K. Vadlamudi and Lee A. Bulla Jr. 2002. Cry1A Toxins of *Bacillus thuringiensis* Bind Specifically to a Region Adjacent to the Membrane-proximal Extracellular Domain of BT-R_1 in *Manduca sexta*: Involvement of a Cadherin in the Entomopathogenicity of *Bacillus thuringiensis.*" *Insect Biochemistry and Molecular Biology*. In press.

Candas, M., B. R. Francis, N. B. Griko and L. A. Bulla, Jr. 2002. Calcium-dependent Cleavage of BT-R_1, a Developmentally Cadherin in *Manduca sexta* Midgut Epithelium. *J. Biol. Chem.* Submitted.

Molecular Characterization of Insect Midgut Toxin Receptors for Circumventing Resistance to Toxins of *Bacillus thuringiensis*

Principal Investigator

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Dr Gamal Osman, AGERI, Egypt

Project Partners

Dr. Lee A. Bulla, Jr., University of Texas, Dallas, Texas

Overall project goal

The overall objective of the research project is to determine the molecular mechanism(s) of insect resistance to the insecticidal toxins (Cry toxins) of *Bacillus thuringiensis*.

Importance of the problem

There are approximately 25 subspecies of *Bacillus thuringiensis* (Bt) that produce an array of parasporal crystalline proteins, known as Cry toxins, whish are lethal to a variety of agriculturally important insects. Because the Cry proteins of the various subspecies have quantifiable specific toxicity to a wide range of different insects and because they pose no demonstrable threat to other non-target organisms or the environment, there is increasing interest in the use of *B. thuringiensis* as a biopesticide. Certainly, the use of Bt has intensified and, during the pest several years, cry genes have been used to transform plants to render them insect resistant. Transgenic plants carrying the toxin genes of Bt have been introduced into the United States and efforts are underway to utilize such plants in Egypt and the Middle East for crop protection. Widespread use of Bt – based bioinsecticides may be causing insects to become resistant to certain Cry toxins. The rise in and expansion of insect infestations due to global warming also in disconcerting and, therefore, it is readily apparent that attention should be paid to improve transgenic plants carrying cry genes as well as those biopesticides formulated with Bt. Designing new and novel Cry toxin molecules or synthetic substitutes derived from Cry toxins should help circumvent the potentially serious problem of insect resistance to Bt.

Project Background

The cotton leaf worm (*Spodoptera littoralis*) is a major problem in Egypt because it destroys horticultural crops such as tomatoes, potatoes and cucurbits as well as cotton. Bt insecticidal toxins effectively control the cotton leaf worm (CLW). Recently, however, the insect has exhibited some

resistance to Bt toxins. Therefore, it is important to gain better understanding of the molecular properties that mediate toxicity to insects such as the cotton leaf worm. Several Bt toxin – binding proteins have been identified in various insects. However, receptor molecules such as BT –R_1 and BTR2 from the tobacco hornworm (*Manduca sexta*) and pink boll worm (*Pectinophera gossypiella*) respectively, have been cloned, sequenced and determined to mediate insect toxicity. The principal investigators in economically important insect pest to Egyptian and American agriculture have also identified several homologues of BT– R1 The toxin - binding site of BT – R_1 has been determined by collaborative efforts between the co-principle investigators, and, determination of its tertiary structure is under investigation. Knowing the mechanism of binding will allow us to a certain the parameters that dictate normal binding versus abnormal or reduced binding in resistant insect species. However, Cry toxin – receptor binding is not the only parameter that contributes in the resistance to the toxin. Therefore, research is underway to determine all factors involved in the resistance phenomenon. The resistance system that is being pursued currently is the Colorado potato beetle in conjunction with Dr. Ed Grafius at Michigan State University. We do not have access to any resistant strains of the cotton leaf worm, but, when they become available, we will compare the potato beetle system to that of the cotton leaf worm.

Achievements

1. Selection for resistant cotton leaf worm by exposing larvae to Cry1C toxin for 25 generations. The resistant strain showed Ten- (10) fold tolerance to Bt toxin than the susceptible strain.
2. Protease activity profiles were examined as well as toxin binding in strain of cotton leaf worm resistant to Cry1C toxin of *Bacillus thuringiensis* strain DI29.
3. Enzymatic processing or degradation of Cry1C toxin showed complete degradation of 130 KDa protein to 65 KDa in the susceptible CLW strain. On the other hand, incomplete degradation of the toxin was observed in resistant CLW strain.
4. The ligand blot showed binding at 107 KDa putative receptor for both susceptible and resistance CLW strains.
5. Detection of BT-R1 homologue using degenerate primers gave a PCR fragment close to the expected size. Cloning of such band is underway.

Ongoing Research

1. Electron microscopy study to show the effects of Cry1C on the histological architecture of susceptible and resistance CLW guts.
2. Study the cross-resistance and stability of resistance between susceptible and resistance strain of CLW.
3. Isolation of receptor gene from both susceptible and resistance CLW strains.
4. Nucleotide sequencing of the two genes to compare the differences at both nucleotide and deduced amino acid levels.

5. Generation of high titer antibodies to these receptor proteins.
6. Conduct study to elucidate the function of these receptors and the role of the midgut proteolytic enzymes in resistance to Bt toxin.

AgBiotechNet

Principal contacts:

CABI *Publishing*, CAB International, UK

Liz Bennett, Publisher,

Dr David Hemming, Editor

Summary

AgBiotechNet aims to deliver current information about biotechnology and biosafety for researchers, scientists and policy-makers worldwide, in both developed and developing countries. The site provides rapid and convenient access to research developments in genetic engineering and updates on economic and social issues as they affect developing and developed countries.

A key element of ***AgBiotechNet*** is the collation and validation of information from diverse sources. The site includes news about corporations, intellectual property rights, technology transfer, biosafety and bioinformatics. The abstracts section presents a searchable database of the world literature. The review article programme and the books section provide in-depth, critical overviews of the field.

AgBiotechNet also features links to related sites, information on patents, detailed spotlight articles on emerging topics, a calendar of events, a jobs section, and an area in which reports and texts from other organizations are published.

Our user statistics continue to show extensive interest from users throughout the world. We are also pleased to report that there have been 2984 direct click-throughs from AgBiotechNet to ABSP, since the site's launch, according to the counter on our site.

The year in brief

This year, ***AgBiotechNet*** transferred its operation from a flat-file system to a database-driven version. This has advantages in reducing admin time, and, eventually, will allow the ability to personalise features. Users will be able to get straight to the material they want, and CABI will be able to generate specific email newsletters for individuals, which will be a major advantage for those with limited connection time.

Our relationship with ISAAA and other developing-country focused biotech organizations has continued, and ***AgBiotechNet*** remains a core essential resource for those who want the latest information on agricultural biotechnology, and especially those concerned with impacts on developing countries.

Content development

The full-text content on ***AgBiotechNet*** has been available to users since January 1999. Available content includes:

News – updated every two weeks; the news section, written by subject specialists covers a variety of topics and is international in scope.

Reviews – updated monthly with specially commissioned articles covering key research issues, economic implications and specific concerns for developing countries.

Highlights from the Review Articles published in 2001 include:

- Modulation of plant organ growth by expansins, the cell wall loosening proteins (Hyung-Taeg Cho)
- Cross-talk between plant defence signalling pathways: boost or burden? (Corné M.J. Pieterse, Jurriaan Ton and L.C. Van Loon)
- Public acceptance: a challenge for the strengthening of biotechnology R & D in Brazil (Leila Macedo Oda and Bernardo E. C. Soares)
- Functional genomics for isolation of genes underlying QTL: disease resistance (David A Lightfoot)
- Do plants communicate with each other via airborne signals? (G Arimura and J Takabayashi)
- Biotechnology and sustainable agriculture (Antoinette M Mannion)
- Why US farmers have adopted genetically modified crops and the impact on US agriculture (Janet Carpenter and Leonard Gianessi)
- Tree genomics in Sweden: a national network of biological and technological expertise (Fredrik Sterky, Björn Sundberg, Rishikesh Bhalerao, Petter Gustafsson, Stefan Jansson, Alwyn Jones, Thomas Moritz, Ove Nilsson, Tuula Teeri, Mathias Uhlen, Joakim Lundeberg and Göran Sandberg)

User statistics from the year show that papers on developing country biotechnology were some of the most frequently downloaded. For example, the review "*Could agricultural biotechnology contribute to poverty alleviation?*" (Charles Spillane) has been downloaded 1,864 times in 2001.

Books – The full text of key books are available as PDF files to AgBiotechNet subscribers. Currently available are:

- ***J I Cohen,*** Managing Agricultural Biotechnology (full text)
- ***P H S Reynolds*** (ed.), Inducible Gene Expression In Plants (full text)
- ***J D Murray, G B Anderson, A M Oberbauer and M M McGloughlin*** (Eds), Transgenic Animals in Agriculture (full text)
- ***R E Evenson, D Gollin, and V Santaniello***, Agricultural Values in Plant Genetic Resources (full text
- ***C L Ives and B M Bedford*** (Eds), Agricultural Biotechnology and International Development (full text)
- ***F H Erbisch and K M Maredia*** (Eds), Intellectual Property Rights in Agricultural Biotechnology, Biotechnology in Agriculture Series, No. 20 (full text)

- ***W H Lesser*** , Sustainable Use of Genetic Resources under the Convention on Biological Diversity: Exploring Access and Benefit Sharing Issues (full text)
- ***C Ritson and D R Harvey***, The Common Agricultural Policy, 2nd edn (full text)
- ***J A Callow, B V Ford-Lloyd and H J Newbury***, Biotechnology and Plant Genetic Resources: Conservation and Use, Biotechnology in Agriculture Series, No.19 (full text).
- ***G Tzotzos***, Genetically Modified Organisms: A Guide to Biosafety. Prepared by the Secretariat of the United Nations Industrial Development Organization (UNIDO) in cooperation with the International Centre for Genetic Engineering and Biotechnology, Vienna, Austria (full text)

Selected extracts from other titles are also available to subscribers.

Links, **Patents** and **Topics** – These sections are all updated on a regular basis and provide comprehensive reference points for existing and new users. In addition, our **Link Exchange** tool allows the user to email CABI with the details of their website so that we can create a reciprocal link to their site. This has had the effect of increasing traffic into AgBiotechNet during 2001.

There are around 447 external web pages linking to AgBiotechNet.

Calendar – The ***AgBiotechNet*** calendar is one of the most comprehensive listings of relevant events, etc., in this subject area.

Reports – CABI *Publishing* continues to work with important organizations generating content in the field. We now have 20 ISAAA *Brief* documents on ***AgBiotechNet***. In November 1999 a series of articles commissioned by IFPRI and edited by Gabrielle Persley were added to the site. It also includes the most recent reports from the National Agricultural Biotechnology Council. The reports include the following topics:

- *World Food Security and Sustainability: The Impacts of Biotechnology and Industry Consolidation*
- *Agricultural Biotechnology: Novel Products and New Partnerships*
- *Resource Management in Challenged Environments*
- *Agricultural Biotechnology and Environmental Quality: Gene Escape and Pest Resistance*

Abstracts database – There are now almost 120,000 records available on the database, with around 1,300 added per month. The search interface was significantly enhanced this year. There are around 12,000 abstracts of biotech-related papers originating in developing countries, representing over 40 different countries.

Listserv – This facility alerts users to new developments e.g. publication of new review articles etc. The number of registered listserv members has grown to 457 since the launch of the service.

AgBio Jobs – this very popular feature covers jobs throughout the world and individual jobs attract hundreds of viewings. Submissions are moderated by the Editor. Access to the service and submission of positions is free of charge.

Directory of Contacts – The newly established directory is receiving many entries from developing country institutions and gives an opportunity to showcase their interests and capabilities, and to

identify collaborators and funding sources. There are directory entries and links for 140 developing country institutes and related organizations.

Access To AgBiotechNet

For the year 2001, access to ***AgBiotechNet*** is available on the following basis:

1. As a paid-for electronic subscription product available from CABI *Publishing*, with corporate, non-corporate (e.g. academic institutions) and personal rates
2. To our partners in ***AgBiotechNet*** including the ABSP staff and its grantees

Usage Statistics: 1 January 2000 to 29 January 2001

Hits: Entire Site (Successful)	1 673 759
Average Per Day	2 757
Home Page	244 915
Page Views (Impressions)	1 304 527
Average Per Day	2 149
Document Views	1 073 624

Visitor Sessions	251 727
Average Per Day	414
Average Visitor Session Length	00:14:14
International Visitor Sessions	19.28%
Visitor Sessions of Unknown Origin	42.8%
Visitor Sessions from United States	37.91

Unique Visitors	106,510
Visitors Who Visited Once	85,316
Visitors Who Visited More Than Once	21,194

Visiting countries

Argentina	Belarus	Bolivia
Bosnia and Herzegovina	Bulgaria	Cambodia
Chile	China	Colombia
Costa Rica	Cote D'Ivoire (Ivory Coast)	Croatia (Hrvatska)
Cuba	Czech Republic	Dominican Republic
Egypt	Estonia	Ethiopia
Fiji	Georgia	Ghana
Guatemala	Hungary	India
Indonesia	Iran	Jamaica
Jordan	Kazakhstan	Kenya
Korea (South)	Latvia	Lebanon
Lithuania	Macau	Malaysia
Mauritius	Mexico	Moldova
Mongolia	Namibia	Nepal
Nicaragua	Nigeria	Oman
Pakistan	Panama	Paraguay
Peru	Philippines	Poland
Romania	Russian Federation	Saudi Arabia
Singapore	Slovak Republic	South Africa
Sri Lanka	Taiwan	Tanzania
Thailand	Togo	Trinidad and Tobago
Turkey	Uganda	Ukraine
Uruguay	Venezuela	Vietnam
Yemen	Yugoslavia	Zambia
Zimbabwe		

Subscriber registrations: 447 institutions and individuals, from the following countries

Argentina	Nepal
Brazil	Pakistan
China	Peru
Costa Rica	Philippines
Cuba	Romania
Hungary	Singapore
India	South Africa
Indonesia	Taiwan
Iran	Thailand
Israel	Turkey
Korea	Venezuela
Malaysia	Vietnam
Mexico	Yugoslavia

AgBiotechNet And Developing Countries

The International Service for the Acquisition of Agri-Biotech Applications (ISAAA) has sponsored access for 20 developing country institutions. This was part of an arrangement that saw CABI training the Managing Editor of ISAAA Knowledge Center for Crop Biotech, and representatives of Biotechnology Information Centers around the developing world. The Knowledge Centre is now up and running, and supplies news on biotech from developing countries to CABI for inclusion in ***AgBiotechNet*** – ensuring it reaches a worldwide audience.

AgBiotechNet hosts information on biotech and developing countries. A hot topic on the subject, incorporating news, reviews, abstracts, and structured links is one of the most frequently visited pages on ***AgBiotechNet***. This service is currently free, and highlights key issues relating to biotech and developing countries in an objective and informed way. It contains around 80 recent news stories, around 50 representative abstracts, links to around 20 reviews, around 100 key organizations, and 35 important external literature sources on agricultural biotechnology in developing countries.

The developing country hot topic:

http://www.agbiotechnet.com/topics/Database/Developing/developing.asp

is now automatically updated with the news, carrying around 10 stories a month, and has accumulated 15,562 visitor sessions in 2001. The page includes links to many key papers on

developing country material.

The UNIDO Biosafety Information Network and Advisory Service (BINAS) and the International Centre for Genetic Engineering and Biotechnology (ICGEB) both license biosafety material from us on an annual basis, including news, reviews and abstracts, which are freely available to all.

CABI continues to publish many books relating to developing countries, with funding provided by ABSP, ACIAR, World Bank, ISNAR,

User Survey

A survey of users and potential users was undertaken in August 2001 to establish opinions on existing features and some new features planned. Some key outcomes were:

- Almost all respondents rated AgBiotechNet a good or excellent publication, and one that they'd definitely or possibly consider using for news, reviews and abstracts.
- Developing country respondents gave very similar answers to those from the developed world.
- The existing format was broadly supported, though changes to allow personalization in what is viewed on the Internet and received by email will enable people to focus on their core interests where appropriate.
- There was support for the newly added directory feature, and for future possibilities such as profiles of key biotechnologists, information on biotech legislation and a discussion forum, with some support for a CV posting database.

The Year Ahead

The year 2002 will see some exciting developments involving AgBiotechNet. CABI is committed in its Information for Development strategy to develop further initiatives relating to GMOs and biosafety, relating to developing countries. The Publishing and Bioscience arms of CABI are working together more closely than ever to ensure that Bioscience project benefit from our extensive publishing programme, including novel information resources like AgBiotechNet. Some of the projects currently being discussed are described below.

Agricultural Biotechnology in China

During the remainder of 2001 and in early 2002, we will be undertaking a pilot project that will involve translating up to five papers from the ***Journal of Agricultural Biotechnology*** published by the China Agricultural University and posting them on AgBiotechNet. We will then gather feedback through questionnaires and invited comments, with a view to regularly publishing an English-language version of the journal and potentially hosting the electronic version on AgBiotechNet as an added value to its users.

Biosafety in Africa

CABI *Bioscience* is spearheading a project in Uganda, funded by Monsanto, which is a subregional (and ultimately pan-African) initiative of capacity building in the implementation and evaluation of GM crop technology. The initiative will identify local needs (infrastructure, equipment and personnel

capabilities of the national implementing organisations) through a number of stakeholder workshops. A comprehensive training programme will develop skills in: conducting local and national needs surveys, engaging stakeholder participation, biosafety protocol development, knowledge of the registration process, impact assessments and farmer participatory training and research. We are currently discussing the role that ***AgBiotechNet*** can play in disseminating information to and about the project.

Journal of Agricultural Genomics

In addition, we are currently in talks with the National Center for Genome Resources in the USA to take over responsibility for the publication of the ***Journal of Agricultural Genomics***. This electronic-only journal has enjoyed some success, but is not considered by the NCGR to be a core activity. We believe that we can, by publishing the journal in both print and Internet versions, create a widely used and valuable resource in agricultural genomics. The Internet version of the journal (or possibly just selected papers) would be featured on AgBiotechNet.

Conclusion

The content and user community of ***AgBiotechNet*** continue to, and we are committed to providing customisation features designed to meet the needs of different users. The feedback accumulated to date and the steady growth in access figures, including countries in the developing world, are very encouraging.

Our future plans will require continued partnerships with organizations like ISAAA, BINAS and ICGEB, and we are particularly keen to continue our relationship with USAID and ABSP. The financial support offered by your organization have enabled us to build a site that is increasingly considered a key information resource for those working in biotechnology, and we are grateful to have received it. In an accompanying paper, we have outlined ways that we hope will facilitate a continued partnership, but we would like to take this opportunity to thank you for your input and support thus far.

Liz Bennett, Publisher

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11 September 2001

Southern African Regional Biosafety Program

Lead Principal Investigator:

Dr. GJ Thompson, ARC-Roodeplaat, South Africa

Project Partners:

Ms. M Koch, Innovation Biotechnology, South Africa

Overall Project Goal

- The overall objective of this program is to build regional policy and technical capacity to support science-based regulation of the development, commercial application, and trade in agricultural products derived from modern biotechnology in the Southern African region.
- The specific objective is to lay the regulatory foundation that will support the field-testing of genetically engineered products (e.g. crops or livestock vaccines) in four of the seven target countries in the SADC region by 2003.

Project Justification

- The Southern African Development Community (SADC) has listed as a policy priority, increased access to biotechnology applications for crop and livestock productivity.
- The private sector will not likely invest in the Southern African region or transfer biotechnology applications such as seeds or livestock vaccines in the absence of government regulatory approval.
- Applications of biotechnology developed through public sector and donor support will also be impeded in the absence of biosafety capacity.
- It remains important to build on the previous foundation of the Regional Biosafety Focal Point (RBFP) in Zimbabwe (ended in 1997) and to continue to build the platform for the harmonization of biosafety implementation in the region. Countries in Southern Africa that have lagged behind in implementation of biosafety structures should benefit from this initiative.
- Many SADC countries plan to ratify the Cartegena Protocol on Biosafety CPB), but few have the capacity to understand the implications of it or to implement its requirements. Most regional delegates to Codex Alimentarius are also ill equipped to participate in the negotiations. Many of the requirements for both Codex and the Cartegena Protocol on Biosafety could be implemented and handled regionally, enabling harmonization together with capacity and cost sharing. It is important for these participants to understand technical issues in order to facilitate legislation, regulations, labeling, regional issues as well as to

harmonize in-country positions / views on agricultural biotechnology. It is also important to equip policy makers to address public concerns and to enable them to communicate these issues to the media in an effective manner.

- Public awareness is becoming a critical factor in the implementation of biotechnology. A 1999 market research survey in South Africa has indicated that there is growing concern about GM foods in the affluent consumer groups, probably fuelled by misinformation presented in sensational popular press.

Background: The current status of Biosafety in the SADC region

- A Regional Biosafety Focal Point (RBFP) for Southern and Eastern Africa was set up in 1995 with a secretariat in Zimbabwe. The funding for the project came from the Dutch Government (DGIS). The Secretariat initiated a biosafety newsletter for the region and established a regional membership of 13 countries, with one affiliate. During the three years in which the RBFP was funded, the Secretariat organized three meetings for member countries that were coupled to three 4-day workshops to build capacity in biosafety. The workshops included case studies to give practical experience to the delegates, most of who had had no exposure to the regulation of genetically modified organisms (GMOs). Also included were discussions on the setting up of national biosafety nodes in member countries and guidelines for drafting regulations and legislation to ensure the safe introduction on the new technology.
- Each member country was invited to send two delegates to each workshop, but sometimes only one delegate attended. Nevertheless, the workshops trained at least three delegates in each member country in the three years. In addition, at least five member countries initiated the drafting of guidelines, regulations and legislation on GMOs as a direct result of the workshops. Funding stopped in 1997 and the RBFP was unable to rise new funding and its activities ceased. The current status of biosafety in the SADC region as well as Africa is summarized in Figure 1.
- Among Southern African countries, only Zimbabwe and South Africa currently have national biosafety regulations. Namibia, Mauritius, Zambia, and Tanzania are in various levels of regulatory development. It is notable that looking beyond legal regulatory development, however, only South Africa has extensive experience conducting regulatory reviews, field tests, and has taken products through to commercial approval. Several Southern African countries are currently examining approval of trials for biotechnology products including:
    1) Heartwater livestock vaccine (Zimbabwe, donor supported)
    2) Insect-resistant cotton (Zambia, private sector)
    3) Potatoes and sugar cane (Mauritius, public and private).

Project Objectives

The SARB Program's primary objective will therefore be to build technical capacity in biosafety in

the Southern African Region and to build the platform for the harmonization of biosafety implementation in the region. This will be done by identifying 7 core target countries in the Southern African region, which are most likely to receive applications for transgenic field trials or commodity imports in the near future. The following countries were identified as core target countries: Malawi, Mauritius, Mozambique, Namibia, South Africa, Zambia and Zimbabwe. A total of 7 major activities were identified to address the objectives of the SARB program.

Activity 1: The establishment of a Regional Working Group consisting of delegates identified by Core Target countries.

The first working group meeting was held in Pretoria, South Africa, during November 2000.

Activity 2: Regional Workshop on Biosafety

This workshop will serve as a general awareness-raising event on biosafety in the SADC region. It is targeted towards legislators/policy makers, regulators, members of biosafety committees as well as delegates to the Cartegena Protocol on Biosafety and Codex. The 3-day workshop will be held in Pretoria, South Africa in March 2001. A maximum of 4 participants from each of 11 SADC countries will be invited to take part in the workshop.

Activity 3: Regional Biosafety Training Course

The purpose of this training course is to train regulators and reviewers (preferably not previously trained) in biotechnology and biosafety issues. This workshop is important for regional interaction, aimed at harmonizing biosafety review policies in the region. The 7-day course will be presented at ARC-Roodeplaat VOPI in June/July 2001. A maximum of 3 participants from each of the 7 Core Target SADC countries will be invited to attend the course.

Activity 4: Journalists/Media Course

Media reporting on biotechnology is increasingly influencing how policy makers develop and implement biosafety regulations as well as public perceptions regarding biotechnology. This workshop aims to provide balanced information on biotechnology and biosafety to key media in target countries. It will also address issues of how policy makers/regulators convey issues of safety and regulation to the media. The 2,5day course will involve 2 participants from each of 7 core target countries.

Activity 5: National Follow-up / In-country Biosafety Training

Pending funding levels and progress by target countries in discussion of regional policy cooperation, national-level biosafety training will be held as a subset of the target countries. The purpose of these national biosafety training activities will be to broaden the range of policy makers with biosafety training to include all members of National Biosafety Committees and other stakeholders such as ministries of trade, industry, farmers organizations, etc. National Biosafety training courses will be presented in the 6 Core Target countries. These courses will vary to meet the specific needs of each country. It is planned to request target country members of the working group to submit proposals on how decentralized in-country funding should be spent in each target country

Activity 6: Risk Assessment Research-Sorghum Gene Flow Case Study

A critical component of biosafety risk assessment and management will be knowledge

about environmental risks specific in the region, such as gene flow from biotech crops to related African species. Gene flow studies on Sorghum will be conducted by VOPI on their research farm near Pretoria to gain risk assessment data and illustrate risk management policy options. The results will provide very valuable information for the African continent.

Activity 7: Core Group Biotechnology Field Trip

The SARB program will also sponsor a site visit to another developing country currently developing GM crops. This will enable delegates to examine other regulatory systems in place or under development. A "Biotech in Action" visit of one delegate from each of the 7 target countries + 1-2 leader(s) will travel to the chosen country (most likely Argentina or China) for 7 days in order to meet with regulatory officials, researchers, farmers, observe field tests, and examine laws and procedures that may be helpful in developing a regional regulatory framework.

Project Progress

During the past year good progress has been made to meeting the objective of SARB. The Work Group is well established but closer communication needs to be established. The Regional training regarding biosafety and risk assessment has been completed and the journalists/ media exposed to this topic. Two countries have completed their in-country training and plans are well underway in the other countries. Field trials to demonstrate gene flow in sorghum are being planned in two countries. Planning of the field trip to China is nearly complete.

Activity 1:Working Group

- Completed following first meeting of the Working Group in Pretoria, South Africa on 19 and 20 November 2000 (see list of current working group members in Addendum 1). Follow-up meetings of the Working Group are planned for 2002 and 2003 to determine the progress made in each country, evaluate the impact of SARB and discuss future ways of assistance and collaboration. Stronger communication links will be established between the SARB coordination office and members of the Working Group through monthly newsletters.

Activity 2: Regional Biosafety Workshop

- Preparations for Activity 2 started in December 2000. A date for the Regional Biosafety Workshop was identified, 28 to 31 March 2001, to be presented at the Head Office of the Agricultural Research Council (ARC) in Pretoria, South Africa.
- A key success factor for Activity 2 will be the nomination of delegates who will participate in the Regional Biosafety Workshop. To ensure that the countries nominate appropriate delegates to attend the workshop, a schedule of visits to Southern African countries was prepared for Dr Brink and Ms Koch. These visits were required to interact at personal level with officials of government departments involved in biosafety in each respective country. The Biosafety situation in each Southern African country is unique and the dynamics of each country's government service must be understood to ensure future participation and buy-in. Members of the working group in core target countries were requested to assist with the logistics for country visits and to arrange meetings with the various departments involved in Biosafety. It was more difficult to arrange visits to non-target countries and

special attention was therefore given to identify key contact persons in these countries.

- A total of 8 Southern African countries were visited during the period 28 January to 28 February 2001 (see short summaries of visits in previous Annual Report) by Dr Brink and Ms Koch. In each country, meetings were held with officials of government departments such as Agriculture, Environment as well as with Biosafety regulators (if available) and members of Biosafety committees. The approach of countries with regard to Biosafety differ considerably and it is also evident that the level of co-ordination between government departments is not always effective. The dynamics of each country that were visited can now be much better appreciated and the specific protocol arrangements with regard to communication and nomination of delegates is clearer.
- A highly successful workshop was held at the ARC Head Office from 28 - 31 March 2001, attended by 48 delegates from 11 countries. (See Activity Report on Regional Biosafety Training Course in Addendum 2).

Activity 3: Regional Biosafety Risk Assessment Workshop

- This course was held from 4 – 10 November 2001 at the ARC Head Office. A total of 21 delegates from the seven core countries representing regulators and reviewers not previously trained, were invited but two failed to arrive. Additional representatives from the Department of Health in South Africa were also invited. (See Addendum 3 for the Activity Report)
- The course allowed for the first practical evaluation of the new ABSP Workbook for Biosafety Officials. The overall opinion was that the course was very successful and the Workbook very applicable. The numerous case studies that delegates had to review and give comments on resulted in strong interaction between them and the course presenters. The Workbook will now form the basis of in-country training of scientists.

Activity 4: Journalists/ Media Workshop

- Preparations for this activity started in January 2001. This activity was combined with a USDA training course to be presented to scientists and policy makers on how to interact and communicate with the media. Activity 4 complimented the USDA course and allowed the media representatives from the core target countries to directly interact with scientists and policy makers.
- The workshop was held at the ARC Head Office from 21 – 23 May 2001. This coincided with the CGIAR mid term meeting to be held in Durban, South Africa. A total of 17 journalists from 7 countries attended the workshop. The full report is given in Addendum 4.
- Future activities in this regard will be coordinated with the AfricaBio/ Rockefeller Project.

Activity 5: National In-country Biosafety Training

- The Work Group teams of each core country were tasked at the Work Group meeting in November 2000 to draw-up a work plan for their country regarding biosafety training. Most of these had been received by August 2001. The plans were reviewed by the SARB management team and recommendations made for revising the plans to each country. Once these had been accepted plans could then be made to implement them.
- The schedule of in-country training is given in Table 1. Dates are tentative and may change

depending of availability of resource persons and outcomes of elections in Zambia and Zimbabwe. Namibia has requested that SARB support an application to conduct a field trial with a GMO as they have other sources of funding for biosafety training.

Table 1: Schedule of In-country Biosafety Training

Country	Dates	No Courses
Malawi	12–16 November 2001	3
Mauritius	April 2002	3
Mozambique	6-7 December 2001	1
Namibia	?	?
South Africa	March 2002	3?
Zambia	May 2002	2
Zimbabwe	August 2002	3

- During 2001 two in-country training courses were successfully completed. Dr Aggrey Ambali was excellent in coordinating the arrangements in Malawi, which resulted in the three workshops being very successful (see Addendum 5).
- Mr Jose Halafo coordinated activities in Mozambique. As this country is possibly the least advanced on biosafety, it was first necessary that he first visit the various provinces to assist in identifying the correct role-players. The workshop dealt with the elementary introduction to biosafety and risk assessment to lay a foundation for them to begin developing their national strategy (see Addendum 6). Problems were experienced in locating a suitable Portuguese speaking resource person. Initially people in Brazil indicated their willingness to assist but this failed to materialize.

Activity 6: Sorghum Gene Flow Case Study

- To date the SARB team has failed to locate a suitable GMO sorghum. Thus discussions have been held with the sorghum breeder at the ARC Grain Crops Institute as to alternative ways of measuring gene flow.
- Plans are now being developed to plant a non-GMO trial at the ARC-Roodeplaat in August 2002. This project will now be led by Mr. Gurling Bothma with Ms. Muffy Koch acting as an consultant.
- Discussions have been held with researchers in Zimbabwe to conduct a similar trial. They have submitted a work plan that is being reviewed.

Activity 7: Core Group Biotechnology Field Trip

- Discussions were held in August 2001 with a local company, China Experience Trade & Tours Company (Pty) Ltd. This company specializes in organizing tours to China and offered their assistance in this matter. They requested contact details of various researchers and institutions that we would like to visit.
- A tentative itinerary was received in January 2002 and is being considered (see Addendum 7). The proposed trip will take place in July 2002. It is essential that invitations be sent out as early as possible in 2002 so that the right people can be selected from each country.

ADDENDUM 1

SARB Working Group

(as of 28 January 2002)

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ADDENDUM 2

SARB Regional Workshop
Pretoria, South Africa
28 - 31 March 2001

Summary report

The Workshop was organised by the Southern Africa Regional Biosafety Program (SARB), with the financial support of USAID.

The aim of this Workshop was to raise awareness for biosafety issues and to discuss the need and possibilities for a regional biosafety initiative.

The Workshop was attended by 48 participants from: Botswana, Lesotho, Malawi, Mauritius, Mozambique, Namibia, South Africa, Swaziland, Tanzania, Zambia and Zimbabwe. Resource persons were present from Ghana, Lesotho, the Netherlands, South Africa, Uganda, the United States and Zimbabwe.

Presentations were given on a number of issues, including:

- An overview of biotechnology,
- An introduction to biosafety risk assessment,
- Requirements of the Cartagena Protocol on Biosafety,
- The regional capacity building project "Implementation of biosafety frameworks in pre-accession countries of Central and Eastern Europe",
- Biosafety management,
- Status of biosafety in Africa,
- Biosafety capacity building in Africa,
- East African experience at regional harmonisation,
- Lessons from attempts at harmonisation of regulation in the SADC region.

Following these presentations, the participants discussed in small working groups the need and possibilities for a regional biosafety initiative.

The findings of the working groups were presented to the plenary meeting, and the following common elements appeared from the reports of the working groups:

- There is a need for a regional biosafety initiative, set up within the SADC structure,
- Such an initiative should be charged with, among others: regional collaboration and co-ordination, capacity building, sharing information and experience, awareness raising, development of regional guidelines, providing a regional web site and clearing house and

providing technical advice,

- The initiative should begin with the formation of a Steering Committee with representatives from each of the SADC countries,

After discussing these findings in detail, the participants agreed as follows:

- An Interim Committee consisting of [to be confirmed] (Botswana),Chaba Mokuku (Lesotho), Greenwell Nyirenda (Malawi), Karuna Mullegadoo for the MSIRI Director (Mauritius), Anabela Zacarias (Mozambique), [to be confirmed] (Namibia), Maryke Herbest (South Africa), [to be advised] (Swaziland), Regina Kapinga (Tanzania), Dorothy Mulenga (Zambia) and Abisai Mafa (Zimbabwe), will develop a proposal for the establishment of a regional biosafety initiative,
- The draft proposal will before 1 May 2001, be circulated to the participants of this workshop, as well as to other interested parties in the SADC countries,
- After consideration of comments received, the final proposal will before 1 June 2001, be submitted to SADC through the SADC Biodiversity Focal Point, which is situated in Malawi,
- The final proposal will also be submitted to UNEP-GEF and other potential donors,
- A copy of the final proposal will be sent to the participants of the workshop and other interested parties in the SADC countries, and will be copied to the SADC Secretariat,
- The Interim Committee will follow the progress of the proposal through SADC.

The participants recommended that SADC take up this initiative as a matter of urgency.

The participants also encouraged the SADC countries to sign and ratify the Biosafety Protocol as a matter of urgency.

ADDENDUM 3

Biosafety Risk Assessment Workshop
Pretoria, South Africa
4 – 10 November 2001

Aim

This activity aimed to train a core group of scientists from each of the target countries in scientific risk assessment.

Attendance and Activities

A total of 21 delegates were invited from the seven core countries. One Mozambiquian and one Malawian did not turn up and South Africa requested additional places for Department of Health staff, who needed biosafety risk assessment training. The final total of 24 delegates was mostly scientists associated with the biosafety working groups or formal structures in each country. One delegate was a biosafety regulator.

The course presenters were the authors of the ABSP Workbook for Training Biosafety Officials (Dr Pat Traynor, Dr Bob Frederick and Ms Muffy Koch), Dr Andrew Matabiri from Zimbabwe and Dr Jane Morris from South Africa.

The programme began with an introduction to biotechnology (for those not active in this field) and a summary of the status of genetic modification (GM) in Africa and the world. The risk assessment training followed the format of the ABSP Workbook for Training Biosafety Officials. The topics included risk assessment, risk management and monitoring and risk communication. The course included risk assessment case studies that were completed in working groups. The programme required homework each evening. A break was included after lunch on day 4 to give the delegates some relaxation time.

This workshop was used to test the new ABSP Workbook for Training Biosafety Officials and the delegates were asked to evaluate both the course and the workbook. The evaluations indicated that the workshop was useful, well organized and of benefit to the delegates. Dr Traynor will assess the workbook evaluations.

Outcome

The delegates were active participants in the workshop and the course generated lively discussion. At the end of the workshop each target country had two or more scientists with comprehensive training in biosafety. This is just the start. The delegates will need to review real applications to practice their skills and build their experience.

The six-day course provided enough time to instill the basics of biosafety and not become tedious or repetitive. However, it is suggested that the first day include a case study exercise to allow some group discussion as a break from the information lectures. This could be achieved by moving the case studies forward.

Next step

The SARB secretariat will forward the names of the delegates to the biosafety focal points in the target countries and ask that they be given an opportunity to practice their skills as national reviewers. In addition, the delegates will each receive a copy of the revised Workbook for their reference.

ADDENDUM 4

Journalists' Workshop on Biotechnology in Africa
Pretoria, South Africa
22 - 23 May 2001

Objectives

This workshop aimed to:

1. Raise awareness of biotechnology among regional journalists,
2. Encourage accurate reporting on biotechnology in the region as a means of raising public awareness about this technology, and
3. Give journalists an opportunity to interact with regional scientists and regulators on the role of biotechnology in Southern Africa.

Programme

The workshop was attended by 17 agricultural and food journalists (delegates list attached) from 7 SADC countries: Southern Africa: Malawi, Mauritius, Mozambique, Namibia, South Africa, Zimbabwe and Zambia. The two-day programme was facilitated by faculty from South Africa (Muffy Koch, SARB), Zambia (Fackson Banda, PANOS) and Zimbabwe (Tinashe Madava, Southern African Research and Documentation Centre - SARDC) and included:

- Introduction to SARB (Muffy Koch)
- Introduction to Biotechnology and GMOs (Muffy Koch)
- The Concerns and Benefits of Agricultural Biotechnology (Muffy Koch)
- Safety checks for GMOs (Muffy Koch)
- Workshop session: Review of genetic modification (GM) media reports (Delegates)
- Ethics in reporting and checking accuracy of information (Tinashe Madava)
- Role of the media in raising public awareness of biotechnology (Fackson Banda)
- Workshop session: Preferred format for material submitted to the media (Delegates)
- Presentations to 39 regional scientists and regulators (Delegates)
- Interview and networking opportunities with regional scientists and regulators (Delegates)
- Field trip – GM cotton farmer and biotechnology research facilities (Delegates; Gurling Bothma and Michele Cloete, ARC)

Outcomes

The delegates participated actively in all the presentations and workshop sessions. They expressed their satisfaction with the clarity of the information sessions on biotechnology and were thorough,

critical and informative in their analysis of four local media reports on biotechnology. This activity required the delegates to use information given in the opening sessions and their feedback indicated that the information had been understood and accurately assimilated.

The presentation on ethics and accuracy of information stimulated considerable input from the delegates. It was clear that they were trained professionals, well aware of their responsibilities as journalists. This session raised the issue of independent press in Africa and gave considerable insight into the constraints of their jobs.

The presentation on the role of the media in public awareness of a new technology was excellent. It established the news worthiness of biotechnology and set parameters under which the media can be effective in raising public awareness. The model PANOS was using to generate inclusive, informed debate on biotechnology in Zambia and Kenya was presented and debated. The discussion focused on some of the methodology shortcomings of the model and possible solutions for these.

The workshop session to prepare feedback for the scientists and regulators, attending the AfricaBio/USDA Communication Workshop at the same facility, was actively persued by all the delegates. They quickly outlined the constraints in preparing articles and listed 'dos and don't' that would facilitate accurate reporting. These suggestions were presented to the delegates of the parallel Communication workshop and generated lively interaction between the delegates of both workshops. The country delegations were identified and the journalists, scientists and regulators were given an opportunity to interact. It was intended that the journalists should interview some of the Communication workshop delegates, but this did not happen effectively. Instead, at the end of a long day, the delegates were content to interact informally and network.

The field trip provided the first opportunity for most of the delegates to see GM crops and to visit a biotechnology research and development facility. This provided a story opportunity and the chance to get visual material for articles. Some of the Journalists visited ARC-Roodeplaat for a "Biotech in Action" excursion. The laboratories where genetically improved plants are produced as well as the glasshouses where these plants are hardened off and propagated were visited. A summary relating to the GM process was presented by Dr Michele Cloete.. The delegates interacted again with the delegates from the communication workshop and this time interviews were arranged or undertaken.

Conclusion

The workshop was successfully completed and resulted in 17 regional journalists with accurate background information on biotechnology, contact details for obtaining information on biotechnology and contacts for regional scientists and regulators who are available to speak about biotechnology in Africa. The inclusion of regional faculty with journalistic expertise greatly facilitated the programme, was appreciated by the delegates and gave additional credibility to the workshop.

Johan Brink and Muffy Koch

24 May 2001

ADDENDUM 5

In-Country Biosafety Capacity Building Lilongwe, Malawi 13 - 16 November 2001

Introduction

The Southern African Regional Biosafety Programme is administrated by ARC-Roodeplaat and was initiated by the Agricultural Biosafety Support Programme (ABSP) based at Michigan State University in the USA.

Various planning and training meetings have been held in South Africa for the seven SADC countries selected to take part in this programme. One of the purposes of the meetings was to establish core groups for each participating country that would establish the biosafety needs for their countries. This was done and the groups then worked out a training programme for their countries, which would best lay a foundation for biosafety issues. The first in-country training programme was held in Lilongwe, Malawi.

The co-ordinator of the Malawi programme is Dr A Ambali, Director, Molecular Biology and Ecology Research Unit, Zomba. The Recourse Team comprised of Dr Ambali, Dr B Frederick (USA), Dr P Traynor (USA), Mr Tinashe Madava (Zimbabwe), Dr G Thompson (RSA) and Mr G Bothma (RSA).

Aim

The general population of Malawi is largely not conversant with the issues of biotechnology, let alone biosafety. The aim of the training was to sensitize various sectors of the public on biotechnology and biosafety. The groups that were targeted were Senior Policy Makers, Directors of relevant fields, Chairman of Parliamentary Committee on the Environment, Media and Scientists. Three groups would receive training pertinent to their fields of expertise. These are: A Media group, Decision-Makers and Scientists.

Attendance and Activities

The first group to receive training was the Media group. Twenty seven representatives from the media attended (Print: The Nation, Daily Times, Malawi News, UDF News, MANA, Information, The Sun, Saturday Post, Chronicles, NAMWA, MIJ, Biotech Update, The Dispatch , photographer; Radio: MBC, FM 101, BBC, Transworld and EAD; TV: SABC)

The second group was the Policy/Decision Makers. Thirty-nine representatives attended and represented Parliament, Agriculture, CAMA, MBS, MES, NAREC, UDF, Fisheries, Monsanto, UNESCO, Bunda College, NRCM, MRA, ARET, DAHI, JICA, EAD, Justice, PHAM, MBERU and Natural Resources.

The third group consisted of Scientists representing the Universities, Polytechnic, Agriculture, Fisheries, Education and Industry. The training for this group was over a period of two days.

See attached programs for each group.

Each group had lectures that were pertinent for the group as well as a time for group discussion. At the end of each of the three groups sessions, time was made available for the groups to ask a panel (comprising of the resource staff) pertinent questions. This lead to further questions as well as lively discussion between the participants.

The Scientist group also had the opportunity to review 3 hypothetical applications for GMO trials (based on real applications) in smaller groups, with report backs to the group.

Outcome

The types of questions raised during the three training sessions confirmed that the level of awareness and understanding by the delegates (in general) of the issues and technicalities of GMO's and Biosafety, were not high. There were exceptions in each group where individuals had a very good grasp of the issues. This training had great value in exposing the delegates to a variety of issues, which could then be discussed in the group. Many of their concerns could also be raised and discussed. During the case studies many non-relevant concerns were raised, but this was due to the selectiveness of the information supplied due to time constraints. The level of awareness with regard to GMO' and Biosafety issue was raised in all three groups. With the ever-increasing possibility of bringing GM products into the country in the near future, through for instance maize imports, getting the correct legislation in place was seen as essential. The groundwork was also laid for the scientists, who would have to do the risk assessment, to have a greater insight into the issues pertaining to assessing the risks.

Having being given a foundation of the principals regarding GMO's the journalists will be able to put more pertinent questions to the role players in the GMO debate. This should also result in more accurate reporting of the issues. No feedback has yet been received from the organizers indicating a difference in the frequency and accuracy of the reporting regarding Biotechnology.

On the whole, the decision making group seemed to have a better grasp of the issues involved and would hopefully be able to make informed decisions. The National GMO legislation was being discussed in Parliament at the time of this training workshop.

Lessons learnt

On the whole the organization and logistics went off well. Dr A Ambali had a competent team working for him and the meetings had been well planned. Presentations should be kept lively, using more pictures and fewer words to illustrate ideas. Communication, especially regarding the presentations needed for the training could have been better. The final programmes of future training workshops should be made available in good time for resource persons to enable them to prepare dynamic presentations.

Next Step

The coordinator of the training in Malawi has been asked to submit an evaluation of the training course given. When this is received a decision will be made on any possible follow up actions.

MOLECULAR BIOLOGY AND ECOLOGY RESEARCH UNIT

Biosafety in Biotechnology Workshops

13 - 16 November 2001 at the Kalikuti Hotel, Lilongwe

Tuesday – 13th November, 2001	
Opening Session Programme for Biosafety in Biotechnology Workshops	
08:15	Participants take their seats
08:30	- Arrival of Guest of Honour - Welcome remarks by *Dr AJD Ambali* - Introductory remarks by *Dr GJ Thompson* – Coordinator for Southern Africa Regional Biosafety Programme) - Opening speech by *Mr C Ndhlovu*, Chief Economicst, Ministry of Natural Resources and Environmental Affairs
09:00	- Group Photograph - **Departure of Guest of Honour**
09:10	- Introduction to Biotechnology – *Dr Bob Frederick* - Biotechnology – *Dr Bob Frederick* - Concerns regarding biotechnology - *Dr Pat Traynor*
10:30	TEA/COFFEE BREAK
10:45	Safety Checks – *Dr Pat Traynor* Regulatory Structures – *Dr Pat Traynor*
11:30	Biotechnology in Africa with particular reference to Malawi – *Dr Aggrey Ambali*
12:15	LUNCH BREAK
13:30	Discussion of Articles on Biotechnology – *Drs Pat Traynor; Bob Frederick & Gurling Bothma*
14:30	Ethics and Role of the Media – *Mr Tinashe Madava*
15:00	TEA/COFFEE BREAK
16:00	General Discussion – *Panel*
16:45	Evaluation
17:00	Payment of per diems

Wednesday – 14th November, 2001	
Biosafety in Biotechnology Workshop for Policy & Decision Makers	
09:00	Introductory Remarks – *Dr Aggrey Ambali, Dr Graham Thompson*
09:10	What is Biotechnology? - *Dr Bob Frederick* How is it being used? - *Dr Bob Frederick*
10:00	Why are biotechnology products regulated? - *Dr Pat Traynor*
10.30	TEA/COFFEE BREAK
11:00	Factors that influence decision making – *Dr Pat Traynor*
11:45	National biosafety systems (cooperation among multiple Ministries) – *Dr Bob Frederick*
12:30	LUNCH BREAK
14:00	Biosafety regulations for Malawi – *Dr Aggrey Ambali*
15:00	Public awareness and consumer concerns – *Dr Pat Traynor* General Discussion
16:00	Evaluation
16:15	End of Session
Thursday – 15th November, 2001	
Biosafety in Biotechnology Workshop for Scientists	
08:30	Introduction E & G
08:45	Introduction to Biotechnology – *Dr GJ Thompson*
09:15	Biotechnology in Africa – *Dr Aggrey Ambali*
09:45	An overview of workbook – *Dr Pat Traynor*
10:00	What is a biosafety review? – *Dr Pat Traynor*
10:30	TEA/COFFEE BREAK
11:00	Approaches to Risk Assessment – *Dr Bob Frederick*
11:45	Scientific issues in Environmental Risk Assessment - *Mr G Bothma*
12:30	LUNCH BREAK
14:00	Case Study 1 Team
15:00	Risk management (lab, greenhouse and field) - *Dr Bob Frederick*
15:30	TEA/COFFEE BREAK

16:00	Case Study 2
Friday – 16th November, 2001	
Biosafety in Biotechnology Workshop for Scientists	
08:30	Monitoring – *Dr Bob Frederick*
09:15	Recommendation Document – *Mr G Bothma*
09:45	Case Study 3 (in groups)
10:30	TEA/COFFEE BREAK
11:00	Case Study 3 (Continuation)
12:30	LUNCH BREAK
14:00	Food Safety - *Mr G Bothma*
14:30	Communicating Risk – *Dr G Thompson*
15:00	TEA/COFFEE BREAK
15:45	Case Study 4
16:45	Closing Discussion

ADDENDUM 6

In-country Biosafety Capacity Building
Maputo, Mozambique
6 - 7 December 2001

Aim

The objective of this activity was to raise biosafety capacity in Mozambique. This target country is the most behind in biotechnology and biosafety implementation, but has tremendous agricultural potential and, therefore, a great need for the technology. The biosafety focal point for the country resides in the department of Environment and has been sensitised to biosafety via the Cartagena Protocol negotiations. Meetings with high-ranking officials in this department in March 2001 indicated that they were both cautious and realistic about the value of genetically modified organisms and the need for a national biosafety framework. The SARB working group for Mozambique includes both the Department of Environment and the Department of Agriculture. The Department of Environment was instrumental in planning and organising the in-country training activity for Mozambique.

Attendance and Activities

The working group identified the need to raise awareness of biotechnology and biosafety within government and national research facilities. Coincidentally the parliament had started an extended debate on biotechnology three weeks before the workshop and this is believed to account for the high attendance and interest generated by the activity. There were 56 delegates from government, research facilities and seven of the 10 provinces. Consumers and environmental organizations were represented.

The first day was dedicated to information sharing with regular question and answer sessions. The organizers asked SARB to direct the information session to lay people in policy development, government and civil society. In fact, the attendance was strongly scientific. A Portuguese-speaking presenter was identified and coached and he gave much of the background information on biotechnology and its relevance for developing countries. Muffy Koch, with the aid of a translator, presented the biosafety information and a local researcher presented human genetics issues. Delegates participated in a safety review activity using case studies of five existing genetically modified crops and all the groups were positive and realistic in their feedback on these examples.

The second day was dedicated to planning a route forward for Mozambique. The delegates were divided into groups, each of which concentrated on a specific discussion area:

1. Policy and Regulation;
2. Public Awareness and Socio-economic Impact;
3. Biosafety;

4. Research and Technology Transfer;
5. Trade, Economics and Intellectual Property.

Each group was asked to debate their topic and present their findings and suggestions under the following headings:

1. National needs and priorities
2. Implementing stakeholders,
3. Strategy,
4. Capacity building,
5. Other.

The group feedback session revealed a positive approach to biotechnology and biosafety and a pragmatic approach to how this should be implemented in Mozambique. The groups identified agencies that should be tasked with implementation and the Department of Environment agreed to see that the outcomes of the workshop would be communicated throughout Mozambique government circles and to relevant stakeholders. The Department of the Environment will be accessing UNEP/GEF funding to set up a national biosafety framework and has asked SARB to be involved in this process.

Outcome

The two-day workshop provided a platform to debate and formulates a way forward for Mozambique's testing and implementation of GM crops and GM technology. The delegates were realistic in their concerns and expectations about the technology and keen to establish the biosafety framework needed to enable implementation and testing. The country has a core group of well-informed officials and scientists who can be called on to assist in the planning and implementation of the next stages.

Lessons learnt

The use of a Portuguese-speaking presenter was a valuable tool. The speaker was well accepted by the delegates who found him credible and his enthusiasm for the technology infectious. His focus on human health issues in addition to agricultural applications also emphasised the scope and importance of the technology for uplifting developing communities.

Difficulties were experienced in communicating with the Workshop coordinator in Mozambique who was frequently out of office as well as setting up financial controls within the country. In future more time must be allowed for the organisation of these workshops coupled with closer communication. If necessary a brief visit to the country should be considered within the last month prior to the workshop to ensure that all is well organised and in place. Also a letter clearing stipulating the procedure of financial responsibility and time lines must be send to the organisers at the conception of the workshop. Although a similar letter was sent they did not adhere to it nor return it.

Lessons were also learnt regarding the transfer of funds between countries. Sufficient time must be

allowed for this. Some of the problems experienced were beyond the control of the SARB management team and lay with our bankers. Steps will be taken to overcome this.

The local USAID mission took an active interest in this workshop. In future the local mission should be informed at an early stage and maybe encouraged to assist with local arrangements.

Next step

Mozambique did not use its full allocation of funding and this will be discussed with the Working Group members to determine how best to use the remaining funding. In discussion with USAID local mission officers, there is an interest in supporting the development of a core agricultural biotechnology competency in the national agricultural research institution so that trials can be carried out with genetically improved crops. Assessing the biosafety of such activities, may be an area where SARB can assist further.

ADDENDUM 7

Core Group Biotechnology Field Trip
China

CHINA EXPERIENCE TRADE & TOURS CO. (PTY) LTD.

2 Hilton Court, Cape Road, Port Elizabeth 2001 Tel: 041-373 3255 Fax: 373 3256

To:	**ARC - LNR**
Attention:	**Mr Gurling Bothma**
Fax No.:	**012-808 0844**
Date:	**16 January 2002**
From:	**Benjamin Zhang**
Subject:	**ARC visit to China**
No. Of Page(s):	**4 (incl. this one)**

Dear Gurling,

Further to our various communication, please find the attached 'proposed itinerary / programme' based on our last year's meeting.

Please let me know if I should 'go ahead' with all necessary reservation with airlines, hotels and confirm meetings with the Chinese side.

As discussed with Dr Xia, Dr Cui and Prof. Huang, everyone agreed that July is a good month to visit them.

Please phone me if you should have any queries at the above telephone number, as well as my cell at 083 676 7445, or fax me at 041-373 3256, or you may e-mail me at chinaexp@iafrica.com or, cett@hinet.co.za

THANK YOU FOR USING CHINA EXPERIENCE SERVICES!

Yours sincerely

Benjamin

CHINA EXPERIENCE

PORT ELIZABETH OFFICE

CC. Mrs. Muffy Kock
CC. Bettie at Ultimate Travel
PROPOSED ITINERARY FOR ARC GROUP TO CHINA

Code: CETT-ARC-020706
Size: 8 to 10 participants
Flying: Cathay Pacific Airways

Day	Date	Description
01	July 06, Saturday	Depart Johannesburg - CX 748 at 12:55 p.m.
02	July 07, Sunday	Hong Kong / Beijing (D.) - KA 908 11:15-14:25 Arrive Hong Kong this morning around 07:50. Connecting flight to Beijing, nation's spiritual heart for the last seven centuries. Beijing is the capital of the People's Republic of China and has a population of over 12 million. It is a favoured city of wide, tree-lined boulevards with both ancient and modern neighbourhoods. On arrival, you are met and transferred to hotel. The reminder of the day is at leisure. Enjoy tonight's Welcome dinner at hotel. Accommodation at Prime Hotel.
03	July 08, Monday	Beijing (B. L. D.) This morning, you'll pay a visit to the Centre of Chinese Agricultural Policy and meet Prof. Huang, the Director of the Centre and his assistance. A business lunch with Prof. Huang and other key personnel of the Centre. In the afternoon, visit to the Beijing Promotion Centre of Agriculture Technology and it's Lavatories of the genetic crops. Dr. Xia will host the afternoon visit.
04	July 09, Tuesday	Beijing / Zhengzhou (B. L. D.) - CZ 3116 10:35-11:25 After breakfast, transfer to airport and flight to Zhengzhou, capital of Henan Province. On arrival, meet and transfer to hotel. After lunch, meeting with experts from 'Centre of Genetic Crops Study' of Henan Province and visit to the experimental fields around Zhengzhou. Dinner. Accommodation at Sofitel Zhengzhou
05	July 10, Wednesday	Zhengzhou / Anyang (B. L. D.) Enjoy a 2-hour drive to Anyang City (about 250 km). Dr. Cui will host the visit to the Cotton Research Institute. A business lunch with the local experts and professionals. In the afternoon, visit to the cotton and maize experimental fields in Anyang. If time permits, visit to the Yin Ruins Museum (with rich collection of pottery, oracle bone fragments, jade and bronze artifacts, etc.). Accommodation at Anyang Hotel
06	July 11, Thursday	Anyang / Zhengzhou / Beijing (B. L. D.) - CZ 3175 18:20-19:20 Return to Zhengzhou by bus this morning. En route, a visit to the Yellow River Scenic Spot and enjoy a cruise on the Yellow River.

		Transfer to airport and flight to Beijing. On arrival, meet and transfer to hotel. Dinner at hotel. Accommodation at Prime Hotel.
07	July 12, Friday	Beijing (B. L. D.) A meeting in the morning with Prof. Huang and Dr. Xia and a business lunch with the Chinese counterparts. In the afternoon, enjoy a visit to Tiananmen Square (the world's largest plaza) and the Forbidden City (the former Imperial Palace). Dinner and followed by acrobatic show.
08	July 13, Saturday	Beijing (B. L. D.) A memorable daylong excursion combines a visit to the soul-stirring Great Wall, winding dragon-like across northern China - only earthly evidence of men, visible from space. Drive along the Sacred Way, guarded by sculpted stone animal both real and fabulous, which are a photographer's delight, to the Ming Tombs. Tonight, enjoy a dinner party at the Peking Duck Restaurant and taste the Number One local dish.
09	July 14,Sunday	Depart for Johannesburg (B.) - CX 749 at 23:40 p.m. Breakfast at hotel. Enjoy a morning visit to the Summer Palace, a breathtaking arrangement of lake and landscape, pagodas and pavilions. A boat ride on the Kunming Lake inside the palace, which was ordered to be built by using the fund planned to build the Chinese navy in the 1900s. Return to hotel and time at your own leisure. In the afternoon, transfer to airport and flight to Hong Kong (KA 993 17:45-21:25). Tonight, board your homeward flight.
10	July 15, Monday	Arrive Johannesburg - around 06:50 a.m.

REMARK: By checking the international flight schedule, we found out that to start on a Saturday and return on Monday morning shall be the best way for above visit. Especially, we can utilize the 5 working days of the week for all business meetings and visit.

Final costs:

Airfare international	R 6,290 per person
Departure taxes	R 268 from SA, R 100 for JNB security tax, R 240 for CX security tax, R 290 for Chinese internal flights departure taxes, R 140 for 'fuel surcharge for all flights into China. Total payable R 1,038 per person
Land arrangements	US$ 130 X 8 days = US$ 1,040 per person based on twin sharing, US$ 360 additional per person for single room supplement
Interpreter	US$ 60 per day X 8 days = US$ 480
Leadership from SA	R 1,000 per person
Visa & handling	R 364 per person

NOTE: Above figures were offered in my previous message to Mr. Bothma (dated the 20th of November 2001).

Booking procedures:

1. A notice in writing from ARC is required to 'go ahead' with all necessary reservation for the visit.
2. The reservation 'cut off' date is 60 days prior to the departure date. However, late reservation can

be arranged and it depends on the availability.

3. A deposit in the amount of R 2,500 per participant is required 60 days prior to departure and balance payment is due to 30 days prior to the scheduled departure date.
4. Payment is required to go through Ultimate Travel in Pretoria.
5. Suggest all participants to purchase travel insurance.
6. The Rand of Exchange will be taken on the date of deposit and balance payment.
7. All visa applications are required to be sent to Ultimate Travel 20 days prior to departure (unless for participants residing outside of SA and will make their own visa arrangements).
8. Final name list of the participants are required 3 weeks prior to departure.

Reg. of company: 97/03811/07 Members: Dr. L. Heunis, Ms. B. Bacon, Mr. B. Zhang

E-mail: chinaexp@iafrica.com

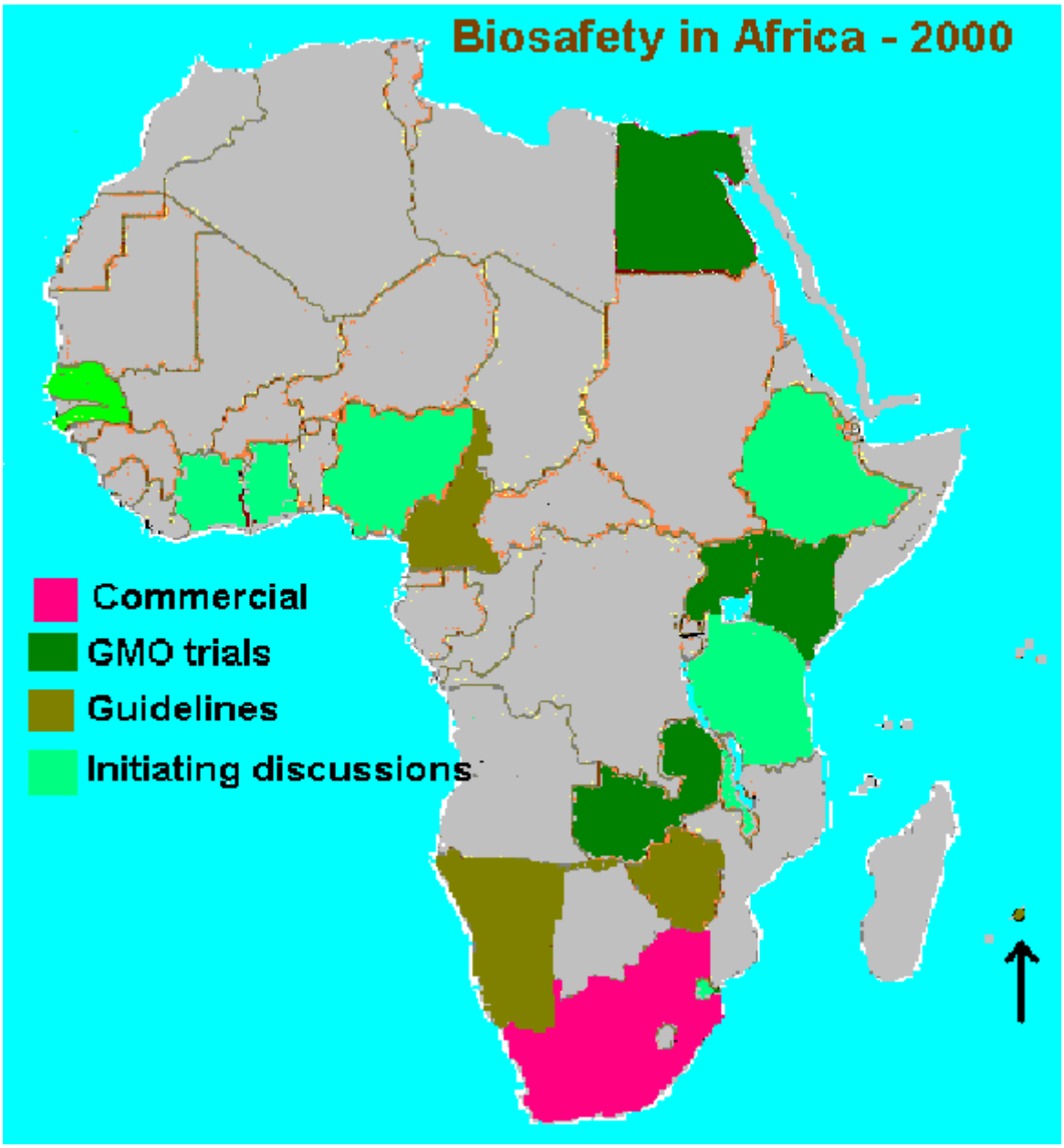

Figure 1. Status of Biosafety Implementation in Africa in 2000 (Source : M Koch)

TRAVEL SUMMARY OF PROJECT PERSONNEL AND PROJECT PARTNERS

PROGRAM: SOUTHERN AFRICAN REGIONAL BIOSAFETY

Institution	Department	Person	Gender	Nationality	Country of Origin	Destination	Purpose	Dates
ARC-VOPI	Biotechnology	Dr J Brink	M	South African	South Africa	Botswana	Consultation	28 - 30.01.01
Innovation Biotech		Ms M Koch	F	South African	South Africa	Botswana	Consultation	28 - 30.01.01
Innovation Biotech		Ms M Koch	F	South African	South Africa	Namibia	Consultation	31.01.01
ARC-VOPI	Biotechnology	Dr J Brink	M	South African	South Africa	Zimbabwe	Consultation	4 - 6.01.01
Innovation Biotech		Ms M Koch	F	South African	South Africa	Zimbabwe	Consultation	4 - 6.01.01
ARC-VOPI	Biotechnology	Dr J Brink	M	South African	South Africa	Mozambique	Consultation	8 - 9.02.01
Innovation Biotech		Ms M Koch	F	South African	South Africa	Mozambique	Consultation	8 - 9.02.01
ARC-VOPI	Biotechnology	Dr J Brink	M	South African	South Africa	Malawi	Consultation	12 - 14.02.01
Innovation Biotech		Ms M Koch	F	South African	South Africa	Mauritius	Consultation	16 - 20.02.01
ARC-VOPI	Biotechnology	Dr J Brink	M	South African	South Africa	Tanzania	Consultation	25 - 28.02.01
Innovation Biotech		Ms M Koch	F	South African	South Africa	Zambia	Consultation	25 - 27.02.01
Univ of Lesotho		Mr C Mokuku	M	Lesotho	Lesotho	South Africa	Workshop	28 - 31.03.01
Council of Science		Dr C Mugoya	M	Uganda	Uganda	South Africa	Workshop	28 - 31.03.01
Univ of Zimbabwe	Biochemistry	Dr I Sitole-Niang	F	Zimbabwe	Zimbabwe	South Africa	Workshop	28 - 31.03.01
		Dr P van der Meer	M	Netherlands	Netherlands	South Africa	Workshop	28 - 31.03.01
US EPA		Dr B Frederick	M	USA	USA	South Africa	Workshop	28 - 31.03.01
BNARI		Dr C Gbedemah	M	Ghana	Ghana	South Africa	Workshop	28 - 31.03.01
SADC		Ms N Mukherjee	F	Zimbabwe	Zimbabwe	South Africa	Workshop	28 - 31.03.01

Institution	Department	Person	Gender	Nationality	Country of	Destination	Purpose	Dates
Int Environ Res		Dr L Reifschneider	F	USA	USA	South Africa	Workshop	28 - 31.03.01
Viginia Tech		Dr P Traynor	F	USA	USA	South Africa	Workshop	28 - 31.03.01
MSU	ABSP	Dr CL Ives	F	USA	USA	South Africa	Workshop	28 - 31.03.01
G/EGAD/AFS		Dr B Pathak	F	USA	US	South Africa	Workshop	28 - 31.03.01
Global Biodiv Inst		Dr J Kilama	M	USA	USA	South Africa	Workshop	28 - 31.03.01
PANOS		Mr F Banda	M	Zambia	Zambia	South Africa	Workshop	20 - 23.05.01
SARDC		Mr T Madava	M	Zimbabwe	Zimbabwe	South Africa	Workshop	20 - 23.05.01
ARC-VOPI	Biotechnology	Dr GJ Thompson	M	South African	South Africa	USA	Meeting	30.10 - 07.11-01
EPA		Dr B Frederick	M	USA	USA	South Africa	Workshop	2 - 10.11.01
Viginia Tech.		Dr P Traynor	F	USA	USA	South Africa	Workshop	2 - 10.11.01
Tobacco Research	Biotechnology	Dr A Matabiri	M	Zimbabwe	Zimbabwe	South Africa	Workshop	3 - 9.11.01
ARC-VOPI	Biotechnology	Dr G Thompson	M	South African	South Africa	Malawi	Workshop	11 - 17.11.01
ARC-VOPI	Biotechnology	Mr G Bothma	M	South African	South Africa	Malawi	Workshop	11 - 17.11.01
EPA		Dr B Frederick	M	USA	USA	Malawi	Workshop	11 - 17.11.01
Viginia Tech.		Dr P Traynor	F	USA	USA	Malawi	Workshop	11 - 17.11.01
SARDC		Mr T Madava	M	Zimbabwe	Zimbabwe	Malawi	Workshop	11 - 17.11.01
ARC-VOPI	Biotechnology	Dr G Thompson	M	South African	South Africa	Mozambique	Workshop	5 - 8.12.01
ARC-VOPI	Biotechnology	Mr G Bothma	M	South African	South Africa	Mozambique	Workshop	5 - 8.12.01
Innovation Biotech		Ms M Koch	F	South African	South Africa	Mozambique	Workshop	5 - 8.12.01
Univ. Natal		Mr T de Oliveira	M	Brazilian	South Africa	Mozambique	Workshop	5 - 8.12.01

PROPOSED TRAVEL OF PROJECT PERSONNEL AND PROJECT PARTNERS

Program: Southern African Regional Biosafety

Institution	Department	Person	Gender	Nationality	Country of Origin	Destination	Purpose	Dates
ARC-VOPI	Biotechnology	Dr GJ Thompson	M	South African	South Africa	Kenya	Meeting	15 - 16.01.02
Innovation Biotech		Ms M Koch	F	South African	South Africa	Namibia	Consultation	February 2002
ARC-VOPI	Biotechnology	SARB Training team*		South African	South Africa	Mauritius	Workshop	April 2002
Innovation Biotech		Ms M Koch	F	South African	South Africa	Mauritius	Workshop	April 2002
ARC-VOPI	Biotechnology	SARB Training team*		South African	South Africa	Zambia	Workshop	May 2002
Innovation Biotech		Ms M Koch	F	South African	South Africa	Zambia	Workshop	May 2002
ARC-VOPI	Biotechnology	SARB Training team*		South African	South Africa	Zimbabwe	Workshop	August 2002
Innovation Biotech		Ms M Koch	F	South African	South Africa	Zimbabwe	Workshop	August 2002
Various		SARB Working Group		Various	Various	Malawi	Meeting	September 2002

* SARB Training team: Dr GJ Thompson(M), Mr GC Bothma(M), Dr M Cloete(F), and Ms R Van Niekerk(F)

Travel report

Attendance at the BTZ Regional Stakeholders Workshop on Biotechnology and Biosafety

Uganda, 10-12 December 2001

Background

The Biotechnology Trust of Zimbabwe is an NGO running a DGIS-funded project to address biotechnology and biosafety capacity building in Southern and Eastern Africa. As such, it has the potential to overlap with SARB activities in the target countries. Dr Johan Brink and Ms Muffy Koch visited BTZ on their trip to Zimbabwe in 2001 and agreed that they needed to maintain contact with this group to ensure that projects were not duplicated and that double funding was not issued for the same activities in target countries.

TZ invited SARB to participate in this meeting that reviewed the BTZ-funded data collection in the region and planned the next phase of BTZ activities. SARB representation was specifically requested to prevent overlap and ensure that the SARB planned activities were well represented at the planning sessions.

Outcomes

Muffy Koch attended the meeting in Uganda. Of the SARB target countries, Malawi, Mauritius, Namibia, Zambia and Zimbabwe gave feedback on the current status of biosafety in their countries. The presentations from Malawi, Namibia, Zambia and Zimbabwe indicated the SARB involvement.

Mauritius did not acknowledge SARB activity and this was challenged. The presenter was the new Biotechnology lead in the Mauritius Department of Agriculture and was unaware of SARB. This was corrected.

Zambia noted a possible call for a moratorium on using GMOs until their legislation was finalized. The impact of this on the country's economics, the practicality of the decision and the possible stagnation of progress it may cause, was pointed out. The meeting agreed that interim implementation procedures were valuable and should be considered in each country that does not yet have legislation. The delegates from Mozambique and South Africa did not turn up, so these reports were not presented.

The meeting allowed consultation with Dr Gopo, who has been contracted by the Namibian government to spearhead biosafety training in that country. He was aware of the dilemma NABA (Namibian Biosafety Alliance) was facing in how to best use the SARB funding and agreed that planning and reviewing a biosafety risk assessment field trial would provide relevant and timely training. He agreed to encourage NABA to consider this approach. It was made clear that approval for this proposal, which is different to the other in-country proposals, will need to be obtained first from the funding agency.

The planning session again identified the need for a regional body to coordinate collaborative biosafety efforts in the two sub-regions – East and Southern Africa. Feasible political structures were again suggested, but it was agreed that parallel to this there is need for a technical implementation process. BTZ would address this in their proposal for the next phase of their funding. This relieves SARB of any pressure to address this function, but we are still requested to draft the Southern Region proposal developed at the SARB Regional meeting in March 2001. The meeting identified ASARECA as a possible regional technical coordinator in East and Central Africa.

Discussions with Zambia indicated that the election date is set for 27 December and they will finalize a date for SARB-funded in-country activities at a meeting soon after this date. The Zambian working group member indicated that they were having problems with the Department of Agriculture member on the SARB working group, as he was acting unilaterally and ignoring guidelines in nominating Zambians to attend SARB activities. This will be taken up with the whole Working Group.

Delegates at the meeting suggested that SARB should coordinate with UNEP/GEF to ensure that the SARB biosafety training capacity is used in the development of biosafety frameworks in those African countries being assisted by the UNEP/GEF project.

Conclusion

It is important for SARB to be involved in regional biosafety planning, if our activities are going to be recognized and not duplicated. These meetings also give an opportunity to look for long term options for SARB once the current project has been successfully completed.

Muffy Koch

13 December 2001

Biosafety Activities Under the ABSP Project

Principal Investigator:

Patricia L. Traynor, Virginia Polytechnic Institute and State University

Overall Project Goals

The ABSP biosafety project is intended to promote the deployment and use of improved crop varieties through short- and long-term technical assistance in biosafety implementation.

Justification

Biotechnology applications in agriculture continue to progress in developing countries on three continents. In ABSP partner countries, the first research products have been field tested and, in some cases, are moving towards commercial release. Other products, derived from institutional research or being imported from elsewhere, are beginning to enter the biosafety system for approval to conduct field tests or larger-scale releases.

Although many of these countries have scientists highly trained in genetic engineering techniques and conventional agronomic skills, there is a widespread shortage of experts trained in biosafety review procedures. This lack of trained biosafety practitioners will likely delay and may even prevent competent and timely review of applications to test new products. Thus there is a strong potential that insufficient biosafety capacity will impede the transfer of this technology into the hands of farmers and growers. Technical training in risk assessment, risk management, and the biosafety review process is the necessary next step for ABSP support.

Project Background

During Phase I, ABSP conducted or supported numerous conferences and workshops that dealt with the need for biosafety policy and the preparation and implementation of guidelines. These meetings were instrumental in catalyzing the formulation of national biosafety guidelines in Egypt (1994), Indonesia (1997), and Kenya (1998). Similar efforts were continued in Morocco (1999). Many of the biotechnology meetings introduced some of the environmental and human health issues raised concerning the release and use of genetically engineered agricultural products. Typically, concepts of risk assessment, risk management, and a step-by-step biosafety review process were presented briefly.

Rationale

ABSP's support of biosafety awareness and implementation has been and continues to be a fundamental component of the ABSP program. The biosafety area is becoming even more important as GMOs developed in collaborative research projects enter the field testing phase and move towards commercial use. Research progress, coupled with increasing requests from multinational companies to import GMO products, are creating a demand for national biosafety systems that are both operational and credible. In the absence of a functional biosafety system, improved GMO crop varieties will not advance through final testing stages and enter the marketplace.

The logical next step for ABSP assistance, then, is to provide timely support for biosafety implementation through short-term technical assistance, and to build capacity for making science-based decisions through technical training in risk assessment and risk management.

Project Objectives with Benchmarks

The current ABSP biosafety project has two main parts. The first is short-term technical assistance, which may include but is not limited to the following tasks:

1. Participate in national and international conferences and workshops;
2. Deliver conference presentations;
3. Consult with policy makers, research administrators, and scientists on matters related to
4. Biosafety (defined broadly), including risk assessment, risk management, risk communication, and public acceptance; and
5. Provide evaluations and reports on biosafety-related topics and materials, as requested by USAID.

The second part is the development of a **Biosafety Review Workbook**, which is to be used in conjunction with a program of in-country technical training workshops. The workbook and course cover:

1. The background and context for biosafety review;
2. Environmental and food safety issues associated with the use of GMOs;
3. Why, when, and where these issues are relevant;
4. Case studies that allow direct experience with the biosafety review process, using a hands-on approach to realistic cases; and
5. Sources of supplementary information and support.

The workbook will be accompanied by a teachers' edition containing instructional materials sufficient to conduct the training course independently. It will be distributed in print and on the CABI

website.

Research Progress

During calendar year 2001, the first objective was met through various conference and training course presentations:

ABSP External Review

Biosafety: An Integral Part of ABSP

SARB Regional Workshop – Pretoria

Biosafety Management: Key to the Environmentally Responsible Use of BiotechnologyManaging Risks in the Lab and GlasshouseManaging Risk in the Field: Options for Limiting Adverse Environmental EffectsAll About Biosafety Review (in 10 minutes or less)

SARB National Workshop – Malawi

A Brief Introduction to Biosafety Review

Concerns Associated with Biotechnology

Scientific Issues in Environmental Risk Assessment

Concerns that Influence Biosafety Decision Making

Safety Checks and Biosafety Regulatory Systems

In addition, I provided assistance in organizing and conducting a regional biosafety training workshop in Pretoria, South Africa

1. 23 participants from 7 countries (Malawi, Mauritius, Mozambique, Namibia, South Africa, Zambia, and Zimbabwe).
2. Opportunity to test the Workbook under training conditions and solicit feedback from trainees

A second workshop was held in Lilongwe, Malawi attended by about 22 participants.

Work on the second objective resulted in an advanced draft of the Biosafety Workbook, which was “field tested” in South Africa and Malawi (Table of Contents given in Annex 1). In addition,

1. The writing team has compiled a list of external reviewers from the biosafety capacity building community and client developing countries;
2. The following production decisions have been made:
   - hire an African editor to ensure the text’s language is clear to African trainees;
   - use of a professional designer for layout of text, tables, photos and illustrations;
   - publish through MSU using a firm previous used for ABSP publications.

Discussion / Implications

The Biosafety Workbook is expected to be published in the second quarter of 2002. Current plans call for it to be freely distributed at no cost to individuals and organizations in developing countries. Partnerships and collaborations in technical training should be pursued with other providers of assistance in biosafety implementation, including BIO-EARN, ISNAR, UNEP-GEF, CIDA, USDA, etc. A web version will be posted as soon as practical.

Travel

The following travel was undertaken in 2001:

March 26-31: Johannesburg, South Africa. Participate in the first meeting of the Southern African Regional Biosafety (SARB) Program.

April 23-25: Washington, DC. Give presentation at the USAID Roundtable "Strategies for Biotechnology in Africa."

June 10-12: East Lansing, MI. Give presentation at the ABSP External Review Panel meeting.

Sept 22-28: Johannesburg, South Africa. Meet with Biosafety Workbook co-authors to advance preparation of the document.

Oct 31- Nov 17: Johannesburg, South Africa. Help conduct a regional SARB training course in South Africa and a national course in Malawi, using the draft Workbook.

Annex 1

Part One: Biosafety in Principle and in Practice

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Section I. Introduction
- A. Rationale and Objectives
- B. Audience
- C. Organization

Section II. Context for Biosafety Review
- A. Factors Affecting Decision Making
- B. Resource Requirements

Section III. Risk Assessment
- A. Approaches to Biotechnology Risk Assessment
- B. Practical Considerations
- C. Scientific Issues for Environmental Risk Assessment
- D. Human Health and Food Safety

Section IV. Risk Management
- A. Risk Management in the Laboratory and Glasshouse
- B. Risk Management in the Field
- C. Managing Pest Resistance to Bt
- D. Other Standard Risk Management Procedures
- E. Risk Management Realities

Section V. Monitoring
- A. Background
- C. Scales of Monitoring
- D. Practical Planning

Section VI. Communicating about Risk and Biosafety
- A. Introduction
- B. Principles of Risk Communication
- C. Risk Communication in Practice

Part Two: Case Study Exercises
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Introduction

Application For Greenhouse Trials With *Ralstonia* Genetically Modified For Biocontrol Of Bacterial Wilt In Potatoes

Application For Greenhouse Trials With Sunflower Genetically Modified For Fungal Tolerance

Application To Run Field Trials With Genetically Modified Bananas Containing A Vaccine To Hepatitis B

Application To Run Field Trials With Cotton Genetically Modified For Increased Resistance To Insect Attack

Application For The Commercial Release Of Genetically Modified Herbicide Tolerant Soya

Application For Commodity Imports Of Genetically Modified Maize

Technology Transfer and Intellectual Property Rights at AGERI, Egypt

Goals:

1. Setup for a permanent office of technology transfer and intellectual property rights for AGERI.
2. Formulate an internal policy for intellectual property rights for AGERI to deal with all aspects of technology transfer and protection of all research finding under the current legal system.
3. Raising awareness among AGERI scientists in the area of IPR and the requirements for patent application according to the Egyptian law.
4. Provide support documents for technology disclosure, material transfer secrecy information transfer and profit sharing.
5. Following up the pending patents at the Egyptian patent office and preparing the necessary documents for patent applications.
6. Continue upgrading the knowledge of AGERI staff regarding the new proposed IP law.

Achievements:

OTTIP office

A permanent office for technology transfer and intellectual property (OTTIP) was set up. The office space has been allocated within the new annex building of AGERI, Which is located cross the way from the main building. All furniture, hardware and software required for full scale running of OTTIP have been transferred to the new office.

Training program:

1. The new IPR officer at AGERI, Ismail AbdelHamid, discussed with Dr. Frederic Erbisch IPR related matters during his visit to Cairo August 2001. During this visit Dr. Erbisch reviewed the IPR policy of the ARC and discussed the possibility of establishing an office for Intellectual Property and Technology Commercialization for ARC.
2. Two participants from AGERI attended a training course during the period September 9 until September 20, 2001 at Institute of international agriculture at Michigan State University (MSU). This training course familiarized them with all aspects of the processes in the technology transfer office. It included the different licensing processes and forms. All current forms such as Material transfer agreement, licenses, and internal policy were discussed and reviewed during the course. The course concentrated on how to encourage the link between AGERI and the private sector.
3. Continue raising the awareness of AGERI staff, especially the newly hired staff, on the

principals, benefits and utilities of intellectual property rights.

Brochures:

Several Brochures related to IPR and technology transfer have been translated into Arabic from MSU booklets. The titles of the brochures are:

1. Creating a company university policies and procedures
2. Protecting your invention
3. Inventorship
4. Material Transfer Agreements

These brochures will be printed and distributed to all AGERI staff and will be included in the training package

IPR activities

1. Meeting with all AGERI senior scientists to discuss the promising scientific work that has potentiality for commercialization. Four promising patents are being prepared for submission to the academy of science patent office.
2. Reviewing new material transfer agreements between AGERI and other institutes and universities.
3. Easy to operate filing system to file and follow up all documents in the office has been established.
4. Filing all possible information on private sector interested in the running projects at AGERI.
5. Following up the pending patents in the patent office by paying the annual fees and replying to any inquiries raised by the national patent office.