



# Annual Technical Report

*January 1 – December 31, 2000*

## Agricultural Biotechnology Support Project

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# Contents

	Page No.
<b>Executive Report 2001</b> _____	1
<b>ABSP Travel 2001</b> _____	24

## APPENDIX -- Technical Reports

### Cucurbits

- 1. Development of disease resistant cucurbit crops using molecular and conventional breeding approaches** \_\_\_\_\_ A1  
Dr. Rebecca Grumet, Michigan State University
- 2. Breeding cucurbits for multiple virus resistance** \_\_\_\_\_ A7  
Dr. Molly Jahn, Cornell University
- 3. Development of virus resistant cucurbit crops using a combination of molecular genetics and conventional breeding approaches** \_\_\_\_\_ A12  
Dr. Atef Sadik, AGERI, Egypt & Dr. Hamdy-El-Doweny, HRI, Egypt

### Potato

- 4. Potato transformation for development of potato tuber moth resistance** \_\_\_\_\_ A27  
Dr. Dave Douches, Dr. Edward Grafius, & Dr. Walter Pett, Michigan State University
- 5. Managing natural and engineered resistance in potato to potato tuber moth** \_\_\_\_\_ A33  
Dr. Magdy Madkour, Dr. Taymour Nasr El-DIn & Dr. Emad Anis, AGERI, Egypt
- 6. Evaluation of Bt-cryV transgenic potatoes on two species of potato tuber moth, *Phthorimaea operculella* (Zeller) and *Symmetrischema tangolias* (Gyen) in Peru** \_\_\_\_\_ A40  
Dr. A. Lagnaoui, Dr. V. Canedo, International Potato Center, Lima, Peru & Dr. D. Douches, Michigan State University

### Tomato

- 7. Tomato transformation for development of geminivirus resistance** \_\_\_\_\_ A48  
Dr. Naglaa A. Abdallah, Prof. Dr. Naglaa Abdallah, Prof. Dr. Mamdouh Idriss, Mr. Khaled Hashem, Mr. Khaled Essam, Mrs. Ghada AbuElheiba, Mrs. Dina El-Amir, Mrs. Lamia El-Gaied, AGERI, Egypt

### ***Drought & salinity tolerance***

8. **Developing drought and salinity tolerant wheat and tomato for Egyptian agriculture** \_\_\_\_\_ A56  
 Prof. Desh Pal S. Verma, Ohio State University
9. **Developing drought and salinity tolerant wheat for Egyptian agriculture** \_\_\_\_\_ A60  
 Dr. Magdy Madkour, Dr. Ahmed Bahieldin, Dr. Ashraf Haider, AGERI, Egypt

### ***Maize***

10. **Development of Asian Corn Borer Resistance in Tropical Maize** \_\_\_\_\_ A71  
 Dr. Terry Meyer, Pioneer Hi-Bred
11. **Maize transformation for development of stem borer resistance** \_\_\_\_\_ A77  
 Dr. Magdy Madkour, Dr. Hanaiya El-Itriby, Dr. Ebtissam Hussein, Dr. Shireen Assem, Dr. Mohamed Abdel Sadek & Dr. Mohamed Eid Saad

### ***Bacillus thuringiensis***

12. **Molecular characterization of insect midgut toxin receptor for circumventing resistance to *Bacillus thuringiensis*.** \_\_\_\_\_ A80  
 Dr. Lee Bulla, University of Texas at Dallas

### ***Other***

13. **Activities of the Office of Technology Transfer and Intellectual Property, Egypt** \_\_\_\_\_ A92
14. **Southern African Regional Biosafety Program** \_\_\_\_\_ A95  
 Dr. Johan Brink, ARC-Roodeplaat, South Africa; & Ms Muffy Koch, Innovation Biotechnology, South Africa
15. **Biosafety activities** \_\_\_\_\_ A108  
 Dr. Pat Traynor, Virginia Tech.
16. **AgBiotechNet** \_\_\_\_\_ A110  
 CABI Publishing, UK

# Introduction

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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 2000.

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

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### **Relocation of Management Offices**

In 2000, the major management challenge was the disruption caused by the arson attack on the ABSP Management Offices. Project staff was re-located to temporary office space in January 2000, and new equipment ordered. Project documents were reconstituted as much as possible, and the project was functioning again within 4-6 weeks. The project suffered further minor disruption in August 2000 when project staff and equipment were moved back into newly renovated offices in Agriculture Hall.

### **Annual Meeting of ABSP External Board of Directors**

The ABSP External Board of Directors held their annual meeting in Cairo, Egypt in May 2000 to coincide with the Commercialization and Utilization of Biotechnology (CUB) Symposium held May 29--June 1, 2000 (see Section on ABSP Networking & Communications later in this Executive Summary for further details on the Symposium). The Board:

- › Congratulated USAID for supporting ABSP, which it considers to be a valuable research and development project, and a model of what can be accomplished in other developing countries.
- › Considered ABSP to be a successful example of how international research and development partnerships can be accomplished between developing countries and advanced research institutions.

The Board also made several recommendations to USAID and ABSP for future activities and programs in agricultural biotechnology and development.

### **ABSP Co-Director Search**

In 2000, the search for a Co-Director for ABSP was initiated. A position description was developed, approved, and posted within MSU and externally via various employment websites, as well as distributed by the ABSP Management. A Search Committee was formed and applications were received and reviewed. An initial short list of candidates was selected, and interviews are scheduled for the first quarter of 2001. The Search Committee intends to select a candidate by the end of the first quarter, 2001, and ABSP anticipated that the Co-Director will begin working by May/June 2001. At that time, the current Director of ABSP will reduce her time to 50%. For the period of a year, ABSP will have 1.5 FTEs at a Director's salary level. This additional cost will come from the core funds of ABSP.

### **Adjunct Appointment**

The Institute of International Agriculture has appointed Dr. Frederic Erbisich as an adjunct professor. Dr. Erbisich, the former head of the Office of Intellectual Property at MSU, will work with ABSP to promote intellectual property and technology management within our collaborating countries as well as develop new linkages with other developing countries.

### **AGERI Management Issues**

ABSP suffered serious delays in the obligation of funds under its USAID/Cairo grant due to USAID/Cairo's insistence that MSU demonstrate cost-sharing under the grant. As this was not a requirement of the contract, and because MSU already cost-shares under the USAID/Washington Cooperative Agreement, MSU could not accommodate this request. As of December 31, 2000 USAID/Cairo had not obligated the next allocation of funds for the ABSP/AGERI collaboration. It is hoped that this problem can be solved in 2001 and additional funds accessed for research programs. Also, USAID/Cairo requested significant management resources to research, source, and justify the purchase of major genomics equipment for AGERI. This was accomplished and ABSP is awaiting funding for this purchase.

### **Subcontracts**

ABSP amended subcontracts with CABI, Virginia Tech, and Cornell. ABSP executed a new subagreement with the Association for Strengthening Agricultural Research in Eastern and Central Africa (ASARECA) for support in developing a regional biotechnology and biosafety program. ABSP is in the process of negotiating contracts with the Vegetable and Ornamental Plant Institute (VOPI), South Africa and Monsanto and expect to finalize those subagreements in early 2001.

### **MSU Biotechnology Survey**

In early 2000 the ABSP Management Team began the process of identifying expertise in agricultural biotechnology at MSU as a first step in the development of proposals for future USAID-funded programs in agricultural biotechnology. The survey was conducted by email with a web-based interface, and sent to faculty in Departments, Institutes and Centers at Michigan State University in all fields related to biotechnology. The announcement that a faculty biotechnology survey would take place was made to Department chairpersons in the fall of 1999, but due to the fire in the ABSP offices the survey itself was sent out in April 2000. As part of the survey, faculty were asked to list their technical and policy areas of expertise, any current or past research projects involving biotechnology, and whether they had any current collaborations or would be interested in developing research links with developing countries. Forty-three faculty members from 18 different units on campus responded to the survey and many of these expressed an interest in future involvement with ABSP in a research, consultancy, or training capacity. As a follow-up to the survey a brown bag luncheon was held in July 2000, for all faculty who responded. The main goal of the meeting was to bring people together to explore the role of agricultural biotechnology and international development at MSU and discuss possible future developments. The meeting was also an excellent vehicle for sharing anticipated USAID biotechnology priorities with faculty.

The information gained from the survey has also been incorporated into the ABSP database. ABSP will undertake a wider survey of international expertise in agricultural biotechnology to identify additional collaborators as part of the process of planning a follow-up program.

### **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. 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, have developed a Scope of Work, and begun the logistical arrangements for the evaluation. It is anticipated that the evaluation will be carried out in May/June 2001.

### **Re-establishment of linkages with USAID**

In October, Dr. Catherine Ives attended the World Food Prize in Des Moines, IA, where she was able to meet with the new Director of the USAID Agriculture and Food Security Office in Washington, DC. Dr. Felipe Manteiga, who was previously with USAID/Port au Prince, was enthusiastic about the ABSP and indicated his interest in learning more about the project. An invitation was sent to Dr. Manteiga to visit MSU, and meet with the Directors of all the USAID-funded agricultural research projects. A meeting was scheduled for January 2001.

### **Future of Agricultural Biotechnology and Development workshop**

ABSP and USAID are currently discussing the purpose, composition, and expected outcomes of a three-day conference/symposia in Washington, DC. The workshop/symposia has been postponed from April 2001 to late 2001 or early 2002. This postponement was due to staff shortages and commitments to project activities. The workshop is still intended to highlight lessons learned from the ABSP and other international biotechnology programs, in order to assist USAID in developing a competitive follow-on proposal.

## **Establishment of a Policy Framework**

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### **Southern Africa Regional Biosafety (SARB) program**

The overall goal of the Southern Africa Regional Biosafety (SARB) program is to conduct regional training related to biotechnology regulation as a means of establishing a foundation for more sound regulatory development and implementation. 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 six SADC countries: Zambia, Zimbabwe, Mozambique, Mauritius, South Africa and Malawi. Namibia may be added at a later date.

The Vegetable and Ornamental Plant Institute (VOPI) of the Agricultural Research Council in South Africa is the lead contractor on the program. Dr. Johan Brink of VOPI has overall management responsibility for the SARB program. Innovation Biotechnology, a private consulting firm headed by Ms. Muffy Koch, is also involved in the development of the program.

On November 19-20, a working group composed of 1-3 representatives from each of the six countries met in Pretoria, South Africa to identify regional needs, review the SARB proposal and develop a work plan for the three year program. Dr. Catherine Ives and Dr. Josette Lewis attended this meeting as resource persons. Working group members were selected based on their key roles in the development of national biosafety guidelines/regulations. The work plans developed included regional activities as well as national follow-up activities to provide more in-depth training and target the broader governmental and private sector constituencies in each country. The Working Group members from each of the core countries drafted national proposals that were presented to the group. Areas of common needs were identified.

In early 2001, Working Group members will hold internal consultations on the SARB program, encouraging national support and participation among key policy makers, identifying suitable candidates for regional activities, and developing national project proposals for in-country activities. Also in early 2001, Dr. Brink and Ms. Koch will make visits to each of the six countries to hold further national discussions and finalize the national components of the program.

### **ASARECA Biotechnology/Biosafety Regional Initiative**

Effort has continued this year in the development of a biotechnology program with the Association for Strengthening Agricultural Research in Eastern and Central Africa (ASARECA). This has included promotion of a regional biosafety initiative, capacity building in biotechnology and biosafety, technical training in risk assessment, and donor coordination activities.

In September 2000, the first meeting of the Biotechnology Working Group of ASARECA met in Entebbe, Uganda. Dr. Pat Traynor (ABSP's Biosafety Specialist) and Dr. Josette Lewis (USAID Biotechnology Specialist and ABSP Project Officer) attended the meeting as resource persons sponsored by ABSP. The purpose of the meeting was to develop the frames of reference for the Working Group and plan subsequent position papers and workshops on biotechnology and biosafety. This ASARECA initiative has, however, suffered from the lack of a half-time coordinator on the project, which has delayed the implementation of workshops and the development of a proposal for donors. ASARECA has advertised for a person to fill this position, but so far no one suitable has been identified. Drs. Josette Lewis and Catherine Ives voiced these concerns to ASARECA during a visit to Kenya in November 2000.

In response to a request by USAID's Africa Bureau on behalf of ASARECA, ABSP produced a report containing an 'inventory' of transgenic crops applicable to the Eastern and Central Africa region. While not exhaustive, this inventory lists transgenic crops that are potentially available for field-testing or commercial release within the next 2-5 years. Primarily this document was prepared for ASARECA in order to provide that organization with some of the background information required to enable them to develop their strategy for biotechnology research in the Eastern and Central Africa region. Our hope is that an additional outcome of this report will be to demonstrate the potential benefits, in the short and longer term, of agricultural biotechnology to Africa.

The report, *An Inventory Of Agricultural Biotechnology For The Eastern And Central Africa Region*, is now available for download from the ABSP website at <http://www.iaa.msu.edu/absp/inventory1.html>. A total of 377 people have visited the web

page to download the report, and many positive comments on it have been received. Hard copies of the report have also been sent to people on request.

### **Grades and Standards Assessments**

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. The general findings of these assessments were:

#### **Kenya:**

- › The fruit and vegetable subsectors are composed of several strata with semi-permeable boundaries, arranged hierarchically in terms of strictness of grading and price.
- › Stronger standards and stricter grades do not appear to be filtering down to the lower strata.
- › The fruit and vegetable subsectors are highly commoditized.
- › A stakeholder's forum should be organized to review and recommend mandates/responsibilities according to areas of specialization.
- › Staff need to be trained or re-trained in order to implement mandates of various organizations.

#### **Malawi:**

- › The commodities with substantially expanded export potential with an improved G&S regime are cassava, cut flowers, pigeon pea, groundnuts, cotton, coffee, Irish potato, Bird's Eye chillies, paprika, ginger and turmeric, and annatto.
- › The capacity of the Malawi Bureau of Standards should be increased.
- › A market linkage strategy that would build upon private sector initiatives should be developed.
- › Technical assistance should be provided to private sector companies seeking to implement Hazard Analysis and Critical Control Point (HACCP) and other G&S applicable to agribusiness.
- › A 'one-stop export shop' should be developed to streamline export processing.
- › A Private Sector Forum of G&S should be established to increase dialogue and dissemination of information regarding global trends in G&S.
- › Government awareness and support for improved G&S through short-term study and fact-finding opportunities in other developing countries should be encouraged.

In November 2000, Dr. Lawrence Busch, Director of the Institute of Food and Agricultural Standards (FAS), attended the *Agriculture, National Resources and Food for Peace Program Officers' Workshop* in Nairobi, Kenya where he gave two presentations on grades and standards, highlighting the findings of the assessment teams in Kenya and Malawi. The interest in G&S issues was substantial.

## Socioeconomic Impact Assessments

Related to the ABSP follow-on process, and in order to clearly show the impact of ABSP, we are in the process of commissioning 2-3 separate socioeconomic impact assessments of activities supported by ABSP. These studies will be on: (1) the potential impact of transgenic potatoes in Egypt and South Africa, and (2) the potential impact of improved cucurbits (squashes/melons) through traditional breeding via extension to the private sector in developing countries.

**Cucurbits:** The Geminivirus diseases of cucurbits are a major constraint to production worldwide. ABSP collaborator, Dr. Molly Jahn at Cornell University has developed multiple virus resistant cucurbit germplasm (cucumber, melon, squash), and built linkages with the private companies in the US and several developing countries to field test and commercialize this germplasm.

ABSP has begun the logistical planning of an *ex-ante* socio-economic impact assessment of this project in two countries in 2001: Indonesia, and South Africa. The multiple virus resistant cucurbits germplasm was sent to the following two private seed companies in October/November 2000 and will be evaluated in the field for disease resistance and agronomic performance in early 2001.

- East-West Seeds, Indonesia
- Alfa Seed, South Africa

A team consisting of a socio-economist, plant breeder, and the ABSP technology transfer coordinator will visit these sites/companies in early to mid 2001 to meet various stakeholders in order to gather the information required for the assessment of the performance and potential advantage of multiple virus resistant cucurbits crops in these countries. Field tests were planted in December 2000, and site visits will be made between March and May 2001. A set of common indicators and methodology will be used across the two countries/sites and a summary report will be developed for USAID and ABSP.

## AGERI Business Plan

The Assessment Team from the University of California at Berkeley conducted and finalized a Strategic Marketing Plan for AGERI. The Assessment Team made the following recommendations for AGERI:

- › Advance a market-drive 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 help shape public opinion of GMOs, but also to learn more about market needs.
- › Promote international standards for intellectual property protection and biosafety regulation to encourage strong relationships with the private sector.
- › Expand the Genetic Engineering Services Unit as an additional source of revenue.

The report also found that:

- › AGERI has a strong scientific culture, but this has led to a lack of market orientation in their research.
- › AGERI does not have strong market knowledge.
- › AGERI has not developed strong links with industry and must link with other members of the value chain in order to access end consumers and determine their needs and future market trends.
- › AGERI should focus its research efforts on maize, and fruits and vegetables if it desires to be profitable.
- › AGERI should focus domestically on input traits.
- › The Genetic Engineering Services Unit (GESU) needs to aggressively market its services and price discriminate to its outside customers.

The next Assessment Team is currently being selected, and will build upon this effort, focusing on the operational management of AGERI.

### **ICGEB Biosafety Workshops**

ABSP sponsored Hisham El-Sheshtawy, the Biosafety Officer at AGERI, Egypt to attend the workshop *Biosafety: Science and Policy in Risk Assessment of Transgenic Organisms: A Case Study Approach* held in Trieste, Italy in March 2000. This workshop was organized by the International Center for Genetic Engineering and Biotechnology (ICGEB) and UNIDO and was directed towards scientists actively involved in biological risk assessment and/or biotechnology regulations. USAID Project Officer Dr. Josette Lewis also attended the workshop.

ABSP sponsored Dr. Mwananyanda Lewanika from the National Institute for Science & Industry Research in Zambia to attend the workshop, *Biosafety: Advanced Research and Procedures: Case Studies for Designated Experts* in Florence, Italy in April 2000. This Workshop was organized by the Istituto Agronomico per l'Oltremare, and ICGEB. ABSP Assistant Director, Dr. Andrea Johanson, also attended this workshop. Following the suggestions of many biosafety focal-points and resource persons ICGEB added this course which was directed exclusively to officers in Governmental Agencies and/or designated experts, working in the area of risk assessment of GMOs at official levels (governments, scientific institutions, private sector etc.). Thirty-eight participants from a wide range of developed, developing and transition economies attended. Most participants were involved in the national biosafety committees in their countries. Individuals from industry, such as Dow, Monsanto and Pioneer, also attended. The workshop was designed on a case study approach with discussion of the basic principles of biosafety tied to a particular case study. Given the high level of expertise present at the meeting, this approach was highly effective.

### **ABIC**

ABSP supported the participation of Dr. John Kakule, Deputy Executive Secretary of the Uganda National Council for Science and Technology (UNCST), to attend the International Agricultural Biotechnology Conference (ABIC) held in Toronto, Canada from June 5-8, 2000. The theme of this conference was *Agbiotech: The Science of a New Century*.

Dr. Kakule writes:

*“The International Agricultural Biotechnology Conference (ABIC) 2000 deliberated on the potential, prospects and challenges of biotechnology in agriculture in a global perspective. Importantly, ABIC 2000 provided an impressive platform for Canadian Universities and Public Research Institutions to present and exhibit innovations in agricultural biotechnology. One would, therefore, wish to suggest that the success of broadening public acceptance of agri-biotech lies in the strengthening and expansion of biotechnology research programs in public research institutions, which are accountable to the public. The efforts of the ABIC Foundation impressed me as focused in this direction. Furthermore, it was clear in ABIC 2000, that agricultural production would need to double to cater for the increasing world population. However, not only food quantity need be a major concern of researchers, but also food quality, which is a key issue for human life expectancy. To developing sub-Saharan Africa, these are critical challenges requiring scientific and technological interventions, a conducive regulatory system and political good will. ABIC 2000 accorded participants the opportunity to appraise themselves with the progress of agricultural biotechnology in the food industry, particularly in the Canadian Provinces of Saskatchewan, Alberta and Ontario. It therefore suffices to say that international interventions for agri-biotech development, such as those of ABIC Foundation, (Canada) and ABSP at Michigan State University, USA, are crucial in promoting food sufficiency strategies in plant-based economies of developing countries.”*

### **Collaborations with BIO-EARN**

Dr. Catherine Ives attended and chaired the Steering Committee for the East African Regional Network on Biotechnology, Biosafety and Biopolicy (BioEARN; <http://www.bio-earn.org>) held in Kenya in November 2000. Bio-EARN is managed by the Stockholm Environment Institute and funded by SIDA/SAREC, the Swedish donor organization. The Bio-EARN program builds technical capacity in Africa in basic molecular techniques, and assists in the development of regulatory policy within its targeted African countries (Tanzania, Kenya, Uganda, and Ethiopia).

Dr. Ives serves as a linkage between Bio-EARN and ABSP, especially in the policy area. In 2000, ABSP and Bio-EARN agreed to share case studies in the development of biosafety training materials, and Bio-EARN requested copies of ABSP's Technical and Impact reports in order to explore the formatting for possible use in Bio-EARN reports to SIDA/SAREC.

### **Collaborations with the International Service for National Agricultural Research (ISNAR)**

Dr. Frederic H. Erbisich, retired Director, Office of Intellectual Property, and Adjunct Professor, Institute of International Agriculture attended ISNAR's management course for selected Asian countries. The course, *Managing Biotechnology In A Time Of Transition*, was held in Kuala Lumpur, Malaysia from 30 October through 10 November 2000. Dr Erbisich presented a paper at the meeting titled *International Collaboration: Intellectual Property Management and Partner-Country Perspectives*. In his talk Dr. Erbisich described the various intellectual property situations, both good and bad, which had been encountered through the ABSP program. This presentation was based upon a publication co-authored by C. Ives, K. Maredia and F. Erbisich. Dr. Erbisich was also involved in case study analyses where he drafted several case studies based on efforts of the MSU Office

of Intellectual Property and led one working group through a detailed analysis of one of these case studies.

### **Biosafety Workbook**

ABSP is working with Dr. Pat Traynor (ABSP's Biosafety expert) in the development of a "***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. Bob Fredericks (EPA), Ms. Muffy Koch (Innovation Biotechnology), and Dr. Andrea Johanson (ABSP Assistant Director) has prepared a first draft of the workbook. The draft has been circulated among the authors and revisions are currently being made. The revised draft will then be sent to selected external reviewers early in 2001.

### **Recent Policy Developments in specific countries**

#### **Indonesia**

In December 2000, the Indonesian Parliament approved the Plant Variety Protection (PVP) Act. This law is based on the UPOV 1991 Convention. ABSP assisted in drafting this new PVP law in 1995. The Indonesian government has also approved the food safety guidelines for genetically modified organisms (GMOs). Through the annual short course in Food Safety, the ABSP project has trained many Indonesian scientists who have since been instrumental in the development of these guidelines. The Ministry of Agriculture recently gave its approval to the limited sale of transgenic cotton.

#### **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 will support this activity through the DAI/APRP policy project.

**Uruguay**

ABSP assisted in developing an outline for an in-country seminar/workshop in IPR, Biosafety and Technology Transfer to build capacity in Uruguay at the request of the Instituto Nacional de Investigación Agropecuaria (INIA). A workshop is planned for Summer/Fall 2001.

**Biotechnology Listserv for USAID**

It was decided in late 1999 that ABSP and the USAID biotechnology officer would create a monthly electronic listserv to engage USAID staff based in the US and in overseas missions in a broader discussion of agricultural biotechnology. The aim of the Listserv was to increase awareness of the issues and opportunities surrounding biotechnology and to explore possible new programmatic approaches on a national, regional and global basis. The listserv was targeted to an audience of government officials and agencies, including 45 USAID employees or contractors. The USAID–Biotechforum Listserv posting were issued bi-weekly from April – July 2000. The topics for the main postings are as follows:

- › Introductory Overview
- › Agricultural biotechnology research and commercial technologies I (Input traits)
- › Agricultural biotechnology research and commercial technologies II (Output traits)
- › Biotechnology and International Agreements
- › Intellectual Property Rights
- › Biosafety, an overview
- › Environmental Issues: Benefits and Risks of Biotechnology in Agriculture
- › Human Health & Food Safety Issues
- › Current USAID biotechnology programs and policies
- › Socioeconomic Issues Surrounding Application of Biotechnology in Developing Countries

A considerable amount of positive feedback was received on the articles and as a package they form the basis for a useful ‘primer’ on agricultural biotechnology. The articles were printed in hard copy and issued by the USAID Project Officer to USAID staff attending the Agriculture, Environment and Private Sector Officers Workshop (AEPS) held in Nairobi, Kenya in November 2000. To encourage confidential debate within USAID the Listserv was restricted to USAID personnel, but the articles will be edited to remove any sensitive information and posted to the ABSP web site early in 2001. USAID’s Africa Bureau is also planning to publish the articles.

**Vegetable Breeding Field Day at Cornell University**

Dr. Karim Mare dia, ABSP Technology Transfer Coordinator, attended the Vegetable Breeding Field Days at Cornell University from August 21-22, 2000. This event was organized by the Vegetable Breeding Institute, and provided the opportunity for vegetable breeders at Cornell to demonstrate their improved germplasm to private companies interested in commercialization of improved vegetable varieties.

Representatives from several companies including Novartis, Sunseeds, Harris Moran Seed Company, Agroflora S/A (Brazil), Starke Ayres PTY Ltd (South Africa) attended the field day. On the first day, the group visited various vegetable breeding research sites near Ithaca, and on the second day, the group traveled to Geneva Research Station.

Collaboration with ABSP has allowed Dr. Molly Jahn, Principal Investigator on the Cucurbits project at Cornell University, to develop new linkages and help distribute disease resistant germplasm to large multinationals and small start-up companies in many countries including Brazil, Indonesia, India, Jordan, Pakistan, Philippines, South Africa, and Turkey.

## ABSP Research Collaborations

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

### Potatoes

**Collaborations with CIP:** The potato project at MSU has an ongoing relationship with the International Potato Center (CIP) to assess the transgenic potatoes developed by MSU researchers. A laboratory study was carried out at CIP to study the larval development of two potato tuber moth species, *Phthorimaea operculella*, and *Symmetrischema tangolias*, which is an important pest of economic importance in potato throughout the Andean region, on the transgenic material from MSU. Results of the detached leaf bioassay showed that mortality of both *P. operculella* and *S. tangolias* was higher on leaves of transgenic Spunta lines. This *cryV-Bt* gene therefore appears to offer a new and additional source of resistance genes for the Andean potato tuber moth. This resistance can be pyramided with other Bt genes for effectiveness toward the development of durable resistance to potato tuber moths and other insect pests. Future work will test mortality of these insects on transgenic tubers, and discussions are currently taking place with CIP to assess the possibility of testing the material in the field.

**Egypt:** In January 2000, under an appropriate MTA, 14 Bt transgenic potato lines were transferred to Egypt for field-testing at AGERI and CIP station in Egypt. This was the fourth year of small-scale field trials in Egypt. A detailed plan is currently being developed for commercialization of Bt potatoes in Egypt that will include environmental data to be collected and analyzed, food safety data to be developed and intellectual property issues to be addressed.

**South Africa:** In December 1999, an MTA was developed to facilitate transfer of 12 Bt transgenic potato lines (tissue culture plantlets) to South Africa for greenhouse and field evaluations. The Agriculture Research Council of South Africa is currently seeking an import permit from the government to obtain these materials from the ABSP potato project at MSU. All the biosafety and IPR legal requirements will be met before the transgenic materials are field-tested in South Africa. Field trials are planned for the fall of 2001.

**Indonesia:** ABSP is still waiting to receive approval from the Indonesian Biosafety Committee for the field-testing of transgenic potatoes developed in collaboration with MSU and the Research Institute for Vegetables (RIV) in Indonesia. We are still hopeful that this can be resolved over the coming year.

## Cucurbits

**Michigan State University:** Research has continued to develop the non-regeneration dependent system for cucurbit transformation. Results have suggested that the electrotransformation procedure that leads to successful production of transgenic progeny may be directly incorporated into the developing floral primordium that is present at the time of electrotransformation. Treatment protocols have therefore been revised to treat older seedlings at a time when they are in the process of initiating floral primordia. Fruit has been now been collected from approximately 200 treated plants and their seeds are now being screened. Pollen tube-mediated transformation has also been tried and seedlings produced from fruit of plants treated in this way are now being screened. The Agrobacterium-mediated transformation system has been used to successfully transform the American cucumber genotypes, Straight 8 and GY14. At least five gene constructs have been successfully introduced as verified by PCR analysis. The Indonesian cultivar Hijau Roket did not regenerate well in the Agrobacterium system.

**Cornell University:** Large scale greenhouse and field-based screens of advanced cucurbit varieties with multiple resistance to virus and other diseases were continued this year in order to identify the lines closest to commercial type which contain the broadest spectrum of disease resistance. A major field day was hosted in Ithaca and attended by 15 seed companies from around the world, and seed from the program has been sent to Africa, Asia and Latin America for trials. 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. Private sector cooperators were identified and are now conducting major trials of ABSP germplasm in South Africa, Indonesia, and Brazil. Trials of this material have also been or are currently being conducted in Jordan and the Philippines. A number of U.S. and European seed companies are also actively breeding with the above material in various locations around the world including Latin America, Mexico, France, the Netherlands, Turkey and India. Syngenta is conducting the most extensive of these trials in early 2001 in Jordan.

**Egypt:** An effective regeneration and transformation system has been developed for the Egyptian cultivars of watermelon, Giza 1 and Giza 21. The important virus, Water Melon Mosaic Virus (WMV) has been successfully isolated, identified and purified, and the coat protein gene of the virus isolated. Evaluation of virus resistance in the multiple virus resistant squash, melon and cucumber lines obtained from Cornell University continued under field conditions at Sids Experimental Station.

Field trials of the ZYMV-tolerant squash lines containing the cp-gene of ZYMV-CT strain (R5) under open field conditions in different locations in Egypt gave promising results. Although there was some variation in infection severity between trial sites, the transgenic plants produced marketable fruits and higher yields than the non-transgenic plants. Initial greenhouse evaluation of ZYMV-tolerant melon lines containing the cp-gene of ZYMV-CT strain (R3) under greenhouse conditions also showed promising results.

## Maize

Significant progress was made in 2000 by Pioneer Hi-Bred in characterizing maize plants that have been transformed with GUS-reporter constructs of the four novel maize promoters referred to in previous annual reports. Samples of transiently transformed maize at AGERI are now showing interesting gene expression results

from these promoters. At Pioneer this year, the stable transformed maize lines with these promoters have been further characterized with regard to relative strength of activity and developmental expression. One or more of them show good promise to serve for expression of *Bt* genes in maize for insect pest control.

## Tomato

Work has continued at AGERI, using several different transformation strategies in order to develop tomatoes with resistance to Geminiviruses. A recombinant DNA construct based on antisense expression of the C1 viral replicator gene has been shown to give a degree of resistance in transgenic plants evaluated until T3 generation. However, the level of resistance obtained from these plants was found not to be stable and was affected by the environmental conditions. Development of a recombinant DNA construct for resistance against TYLCV based on virus-induced expression of cytotoxin gene gave some success, and after three months from transplantation in the T3 generation, only 57 plants out of 180 (31.6%) showed typical TYLCV symptoms in glasshouse containment studies. Transgenic plants engineered with coat protein gene in the sense orientation showed no viral resistance; therefore, this strategy is not recommended to obtain resistance against geminiviruses.

## Molecular characterization of insect midgut toxin receptors

Research at UT Dallas continues to investigate the molecular basis of insect resistance to the *Bt* toxins. Widespread and extensive use of Cry toxin formulations to control insects has increased the likelihood for development of resistance to *Bt*. Cry toxin degradation by proteolysis has been postulated as a possible mechanism for insects to evade deleterious effects of Cry toxin, and therefore protease activity profiles were examined as well as toxin-binding in a strain of Colorado potato beetle resistant to the Cry3A toxin of *B. thuringiensis subsp. tenebrionis*. Specific proteolytic enzymes were found to be present in midgut extracts and brush border membrane vesicles of the resistant strain that were absent in the susceptible strain. Aminopeptidase activity associated with the vesicles from insect midgut was higher in the resistant strain than in the susceptible one. Enzymatic processing or degradation of Cry3A toxin did not differ in these strains and, apparently, is not a factor. However, the vesicles from the resistant strain bound approximately 60% less Cry toxin than vesicles from the susceptible strain. Also, saturation kinetics of toxin binding in the susceptible strain is 30-fold greater than in the resistant one. In vivo experiments confirm that the susceptible strain retains more toxin in its midgut than does the resistant strain which excretes more toxin than does the susceptible strain. Histological examination revealed that midgut epithelial cells from the susceptible insect are devastated by *Bt* toxin action whereas cells from the resistant insect retain their structural and functional integrity. Resistance to *Bt* toxin therefore involves not only decreased toxin binding and increased excretion of toxin but also changes in the composition and activity of midgut proteolytic enzymes, especially elevated aminopeptidase activity.

## Drought tolerance

Collaboration between AGERI and Ohio State University has begun by transforming immature embryos of Egyptian and American bread wheats with genes for salt and drought tolerance obtained from Dr. Verma at Ohio State University. Using other

genes at AGERI, many putative transgenic plants of both Egyptian and American cultivars have been obtained. In addition, regeneration and transformation efficiencies have been increased by shortening the selection period, and bombarding young immature embryos. AGERI have succeeded in transforming Egyptian wheats (Giza 163 and Giza 164) with salt-related genes (i.e., mtlD and fructan-accumulating, respectively) and achieved good expression for the first gene under stress condition. The other gene is now being evaluated to test the efficiency of using it in conferring salt tolerance to the Egyptian wheat cultivar Giza 164.

## High Beta-Carotene Mustard Oil

Dr. Catherine Ives traveled to Davis, CA in March in order to discuss the science and management of a proposed collaboration between Monsanto Company (St. Louis, MO), the TATA Energy Research Institute (TERI) and ABSP/MSU in order to develop high beta-carotene mustard oil for use in India. These mustard varieties will provide a food-based approach to reducing Vitamin A deficiency in India. ABSP/MSU will provide management and fiscal oversight to this effort, with Monsanto providing technical oversight. ABSP will also provide support for policy issues arising from this work, including public acceptance and linkage development to the health system. Funds for this effort were released by USAID to MSU in October 2000. Negotiations have been ongoing to finalize a work plan and budget for Monsanto and TERI. It is hoped that we will have a signed subagreement in early 2001.

## ABSP Networking & Communications

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### Egypt Symposium

The CUB Symposium, held May 29-June 1, 2000 in Giza/Cairo, Egypt was a joint effort of the Agricultural Genetic Engineering Research Institute (AGERI) and the Agricultural Biotechnology Support Project (ABSP). This unique, collaborative symposium was funded by USAID/ATUT with support from AGERI and the Agricultural Research Center (ARC) in Giza, Egypt. Thirty participants traveled to the symposium from the U.S. and Europe. The ABSP management team and researchers from Michigan State University attended the symposium along with ABSP sub-contract researchers from several U.S. universities, U.S. government officials, non-profit organizations, and private industry from the U.S. and Europe. About 40-50 Egyptian researchers, institute and government officials and Egyptian private industry representatives attended the 4-day symposium. The ABSP External Board of Directors also held the ABSP Annual Review Meeting in conjunction with the symposium to view, first hand, the collaborative biotechnology efforts in Egypt. *For further details on the symposium refer to ABSP Quarter 2, 2000 **Linkages** newsletter at <http://www.iaa.msu.edu/absp/links00-2.html>*

### ABSP profile raised

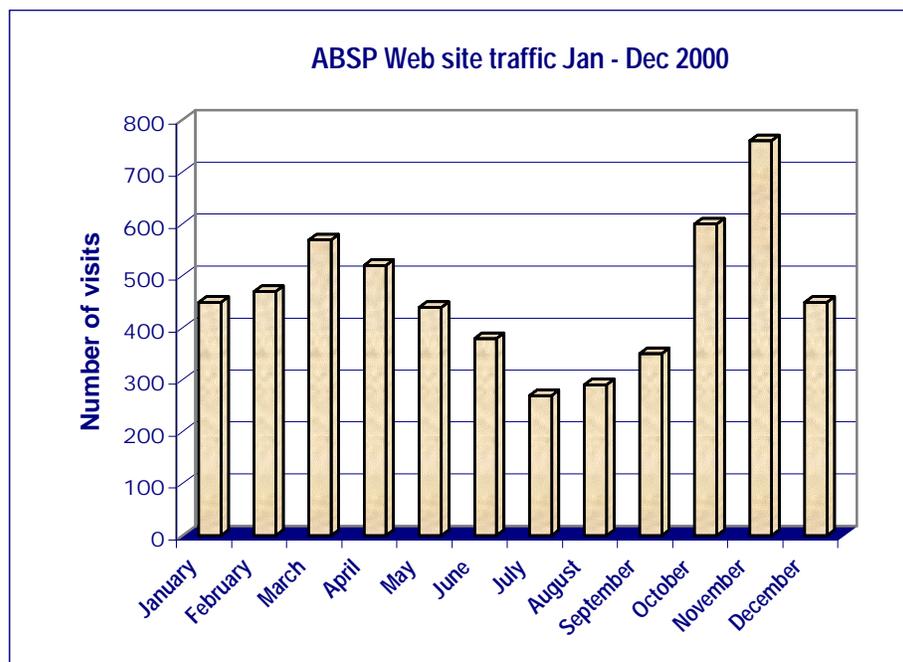
One positive outcome of the New Year's Eve arson at the Management Offices of the ABSP was the opportunity to communicate to the general public the need for and goals of the ABSP via television, radio and print coverage. The Director of ABSP gave numerous interviews to various organizations including CBS Evening News, the New York Times, the Los Angeles Times, the Detroit Free Press, the Lansing State Journal, MSU Alumni

Magazine and MSU State News, and local radio stations in Michigan. In addition, the Director has been involved in on-campus discussions on agricultural biotechnology and is increasingly asked to speak on agricultural biotechnology and its importance for developing country agriculture. The coverage has overall been very positive and has raised the profile of the project.

### Development of ABSP's World Wide Web pages

The ABSP Website continued to receive an increasing amount of traffic during the past year. There have been over 7,000 visitors to the Website since August of 1999, with the average monthly number rising to a high of over 700 in November 2000. This coincides with the posting of the Agricultural Biotechnology Inventory for Africa on the web pages, and also the submission of the web site details to a range of internet search engines. Many of the major agricultural biotechnology websites now have a link to the ABSP site from their pages.

A section on technology transfer has been added to the site, with further details concerning Intellectual Property Rights (IPR), and the way in which ABSP and MSU manage technology transfer issues. Visitors are now able to download forms from the site, including the Invention Disclosure Form, the Biologically Active Material Transfer Agreement and an example of a Research Agreement. Forms are available in both Microsoft Word and PDF formats.



### Development of AgbiotechNet

*AgBiotechNet* publishes current information about biotechnology and biosafety for researchers and policy makers worldwide. The site provides rapid and convenient access to research developments in genetic engineering and updates on economic and social issues. The contents and user community of **AgBiotechNet** have continued to grow since launch in January 1999, and are starting to involve novel ways of tailoring it 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. Discussions with ISAAA have been fruitful, leading to an extension in access and the depth of coverage of developing world issues. The average number of user sessions per day since the launch in January 1999 increased from around 80 in January 1999 to over 300 in December 2000.

*AgBiotechNet* now hosts an information section 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 entirely free service highlights key issues relating to biotech and developing countries in an objective and informed way.

There are now over 80,000 records available in the abstracts database, with around 1300 added per month – a major increase over 1999 levels. The search interface will be significantly enhanced in March.

CABI *Publishing* continues to work with important organizations generating content in the field, and *AgBiotechNet* now contains 20 ISAAA *Brief* documents, a series of articles commissioned by IFPRI, and the most recent reports from the National Agricultural Biotechnology Council. The reports include the following topics:

- ◆ **Will global agbiotech pay? A review of sales trends** (Richard Leech) ABN 054
- ◆ **GMOs in developing countries** (Alain Weil) ABN 052
- ◆ **Could agricultural biotechnology contribute to poverty alleviation?** (Charles Spillane) ABN 042
- ◆ **Genetically modified plants: developing countries and the public acceptance debate** (John H. Skerritt) ABN 040

ABSP's current *AgBiotechNet* subscribers include:

- › Zamarano Escuela Agricola Panamericana, Honduras (added in 2000).
- › Kenya Agricultural Research Institute, Kenya (added in 2000).
- › Institute of Cell Biology and Genetic Engineering, National Academy of Science, Ukraine (added in 2000).
- › National Bureau of Plant Genetic Resources, India.
- › Kawanda Agricultural Research Institute Library, Uganda.
- › Uganda National Council for Science and Technology (UNCST), Uganda.
- › Agricultural Genetic Engineering Research Institute (AGERI), Egypt.
- › Ethiopian Agricultural Research Organization (EARO), Ethiopia.
- › ARC-Roodeplaat V.O.P.I, Republic of South Africa.

## **ABSP Database Development**

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 continues to expand and develop the contacts and upgrade and improve the database management system. This year we have included an improved contact

database of expertise in all fields of agricultural biotechnology. The database currently contains over 900 contacts.

### **LINKAGES Newsletter**

The first electronic *ABSP LINKAGES* newsletter was distributed in April of 1999 to about 350 contacts. The newsletter 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. The 4<sup>th</sup> quarter 2000 *LINKAGES* will be distributed to 935 contacts, almost triple the size of the first electronic newsletter distribution. Contact email addresses are exported from the ABSP database for each newsletter mailing, which keeps the distribution of the newsletter as current as possible. *LINKAGES* is also posted to the ABSP web site under ABSP News. Unsolicited comments on the newsletter have been very positive.

### **MSU Training Courses**

ABSP has continued to support individuals to attend MSU courses on Food safety and Intellectual Property Rights.

#### **Intellectual Property Rights (IPR) and Technology Transfer Internship program**

In 2000, ABSP sponsored four individuals to attend this program, held at Michigan State University from July 9 -14, 2000. The internship program provided hands-on experience to international participants in the day-to-day handling and management of intellectual properties in various settings and was attended by 16 international participants from 9 countries. ABSP partially or fully sponsored the following participants to attend:

- ◆ **Mr. Motaz A. Moniem**, Agricultural Genetic Engineering Research Institute (AGERI), Giza, Egypt
- ◆ **Dr. Tantono Subagyo**, Intellectual Property and Technology Transfer Office (KIAT) - AARD, Bogor, Indonesia
- ◆ **Dr. Arvind Duggal**, Department of Biotechnology (DBT), New Delhi, India
- ◆ **Dr. Suresh Chandran**, National Institute of Immunology, New Delhi, India

One participant from the Philippines spent an additional week with Dr. Erbisch to learn more about handling of intellectual properties, in particular, the management of new crop varieties. The training this individual received has helped in solving several intellectual property situations at his institution.

#### **Food Safety Short Course**

ABSP sponsored three individuals to attend the International Food Safety Course held at Michigan State University from July 16 -21, 2000. The course was attended by 17 international participants from 11 countries and provided hands-on experience to international participants in food safety policy development, risk analysis and program implementation to ensure a safe food supply for the global community. During the course, a special discussion session was held on food

safety issues associate with Genetically Modified Organisms (GMOs) and other emerging issues in food safety. ABSP partially or fully sponsored the following participants:

- ◆ Ms. Hamza Badaweya, Food Technology Research Institute, Agriculture Research Center, Giza, Egypt
- ◆ Mr. Gasilan, Indonesia, Directorate General Food and Drug Inspection, Ministry of Health, Jakarta, Indonesia
- ◆ Mr. Bambang Dwiymoko, National Quality Control Laboratory, Food and Drug Inspection, Jakarta, Indonesia

### **Philippines Biotechnology Assessment**

In June, Dr. Ives led a team composed of US government officials, private sector consultants and university representatives to the Philippines for a three-week assessment of the current status of agricultural biotechnology research and commercialization. The goal of this effort, supported by USDA, was to provide to the Government of the Philippines with general recommendations for the development of an agricultural biotechnology program. Seven million dollars of PL480 funds will support research and policy efforts.

Recommendations for the Program were made for both policy and research components, with the primary recommendation being an emphasis on institutional support, including equipment grants, followed by research grants, followed by training opportunities.

In the policy area, recommendations include to:

- › Re-examine of the current biosafety guidelines, with an emphasis on the costs of implementation to local innovators and incorporation of new information and experience
- › Support policy research on the implementation of regulations, including an analysis of food safety requirements, and the cost of mandatory labeling
- › Set priorities for biotechnology research projects based on comparative advantage of Filipino R&D institutions, potential economic returns, linkage with downstream users and development of multi-disciplinary projects
- › Develop a consistent intellectual property policy for public R&D institutions and conduct an education and training program for researchers in the management of intellectual property
- › Encourage research institutions to participate in the formulation and implementation of regulatory policy

In the research area, recommendations include:

- › Primary support for institutional support, including equipment grants (60% of total funding)
- › Develop two major core facilities at UPLB and PhilRice
- › Secondary support for research grants, totaling 30% of overall funds
- › Support short-term training, especially for regulators, and develop information resources for these individuals.

### **Intellectual property policy assistance to the Bean/Cowpea CRSP**

With ABSP assistance and consultation, the USAID-supported Bean/Cowpea Collaborative Research Support Project (CRSP) developed an IP policy – the first time a CRSP project has institutionalized an IP policy with their US and overseas collaborators. The Bean/Cowpea CRSP Technical Committee reviewed the draft policy and it is currently under consideration by the Bean/Cowpea CRSP community. The adoption of a consistent IP policy will assist the Bean/Cowpea CRSP in meeting federal obligations and provide clear guidance on the management of intellectual property rights within the CRSP.

### **Public Perception of GMOs in India**

ABSP partially supported the visit of Tomiko Yamaguchi, a doctoral candidate in sociology at MSU, to India from July-September. Ms. Yamaguchi conducted a preliminary study for her doctoral thesis exploring the current public discussion surrounding GMOs in India. She collected literature and conducted interviews with a wide cross-section of interested parties in order to evaluate public perceptions regarding the value and risk of GMOs and how GMOs may impact their lives in the future. She is also reviewing the current regulatory status of GMOs in India, which will be beneficial to ABSP as it moves forward with its development of Vitamin A enhanced mustard oil through ABSP's Monsanto/TERI collaboration.

## **ABSP participation in conferences & workshops**

### **Workshop on Transgenic Potatoes for the Benefit of Resource-Poor Farmers in Developing Countries**

Dr. Walter Pett, Department of Entomology, Michigan State University and ABSP researcher on transgenic potatoes, attended the international potato workshop held in Manchester, United Kingdom from June 5-9, 2000. The workshop, sponsored by the British Department for International Development (DFID) Plant Sciences Research Program, UK; DFID Crop Protection Program, UK and the International Potato Center (CIP), Peru, was well attended. Representatives from Kenya, Uganda, South Africa, Argentina, Cuba, Bolivia, Mexico, Colombia, Peru, China, India, Nepal, the USA and the United Kingdom presented country profiles on the first day of the workshop. The second day, technical issues and concerns of transgenic potatoes was the focus of the presentations including gene flow, pyramiding genes, transgenic expression, gene flow to other potatoes and wild species and insect control. On the final day of the workshop, policy issues in the deployment of transgenic plants in developing countries was the focal point, followed by panel discussion and working group sessions.

### **International Workshop on Biosafety and Food Safety of Genetically Engineered Products**

Dr. Karim Maredia, Technology Transfer Coordinator, ABSP, traveled to Indonesia to present a paper on "Capacity Building in Biosafety: Case Study of USAID-ABSP" at the held in Jakarta, Indonesia 1 & 2 February 2000. The workshop was co-organized by the Indonesian Society of Agricultural Biotechnology and co-sponsored by the Agency of Agricultural Research and Development, ABSP, Asia-Pacific Crop Protection Association and Pioneer Hi-Bred International. The theme of this workshop was the safe use of genetically engineered products to support agricultural productivity. Over 300 participants listened to topics related to biosafety issues that

included food safety considerations, Indonesian experiences on biosafety capacity building and field-testing and discussion on public awareness and harmonization of biosafety and food safety regulations. Dr. Mare dia met with Dr. Muhammad Herman, Biotechnology/CRIFC, Mr. Prijanto Santoso, USAID/Jakarta and Dr. Achmad Fagi, AARD. A visit was also paid to KIAT, the new intellectual property rights and technology transfer office in Bogor, to meet with Dr. Tanton o Subagyo on the current activities of KIAT.

#### **World Food Prize Meeting**

In October, Dr. Catherine Ives attended the World Food Prize in Des Moines, Iowa. During this symposium, Dr. Ives was a participant and session leader in an informal meeting to discuss the need for, the function of, and the development of a technology transfer foundation or clearinghouse to access proprietary biotechnology tools for use in developing country agriculture. The purpose of the clearinghouse would be to have easily accessible databases developed that clearly describe the IP status of various research tools and target genes, and the status of their availability for various countries. In addition, the clearinghouse could serve as a negotiating arm to access proprietary materials from both the public and private sector in the developed world. Dr. Ives has been invited to speak at a follow-up to this meeting in early 2001.

### **Invited presentations**

#### **International Food and Nutrition Conference 2000**

From October 8-10, Dr. Catherine Ives attended the International Food and Nutrition Conference 2000 at Tuskegee University. The theme of the conference was *Food for the New Millennium: Innovations in Nutrition, Food Safety and Biotechnology*. Dr. Ives gave a presentation entitled *Biotechnology 101: An Introduction to the Science and Policy*.

#### **MSU Animal Science Department**

On September 18, Dr. Catherine Ives was an invited speaker for the MSU Animal Science Department's seminar series. The title of her lecture was *Biotechnology and Development*.

#### **Green Belt Movement, Kenya**

During her visit to Kenya in November, Dr. Catherine Ives met with the Green Belt Movement, an NGO that focuses on environmental conservation and community development. The GBM recently initiated a core program in food security, focusing on enhancing farmers' knowledge and productivity, using indigenous crops. Dr. Ives gave a presentation on agricultural biotechnology, and how it might address constraints in African agriculture.

#### **Association of American Warehouse Control Offices**

On April 20, Dr. Catherine Ives spoke at the annual Association of American Warehouse Control Offices meeting in Lansing, MI. The AAWCO aspires to sustain and augment the network of association states, provinces and affiliates of grain warehouses. Dr. Ives spoke about the ABSP and biotechnology in development.

#### **The Challenges Of The Livestock Revolution Workshop**

Dr Karim Mare dia attended the workshop *The Challenges Of The Livestock Revolution* at USAID, Washington, D.C. (November 14 –15, 2000), and gave a short presentation on *Issues and challenges involved in Technology Transfer; Capacity Building in IPR, Biosafety and Food Safety*.

**Workshop On The Impact On Research And Development Of *Sui Generis* Approaches To Plant Variety Protection Of Rice In Developing Countries, Philippines**

Dr Erbisch presented a paper titled *Challenges of PVP Administration: How a Public Agricultural Research Institution Protects Its New Plant Varieties and Markets Them* at an International Rice Research Institute (IRRI) meeting in the Philippines on the handling of new crop varieties. Dr. Erbisch met with representative from at least twelve different countries to discuss intellectual property management situations, and Dr. Erbisch with several IRRI representatives to discuss handling of intellectual properties of IRRI. The presentations were published recently as a limited proceedings titled "*Plant Variety Protection for Rice in Developing countries: Impacts on Research and Development*" and it is available from IRRI.

**Biotechnology Research and Policy: Needs and Priorities in the Context of Southeast Asia's Agricultural Objectives, Thailand**

Dr Erbisch presented a paper titled *IPR In Southeast Asian Biotechnology*, which dealt with intellectual property rights in Southeast Asian agricultural biotechnology at the Regional Conference on Agricultural Biotechnology in held in Thailand. Dr. Erbisch met with representatives from most of the Southeast Asia countries. The presentations from this meeting will be published in the near future.

**Scientia 2000 Intellectual Property in Academic Institutions, Brazil**

Dr Erbisch presented a paper titled *Technology Transfer and United States Universities: An Evolution in Progress* at "Scientia 2000 Intellectual Property in Academic Institutions", held in Brazil. The presentation will be published in book format and will be distributed to colleges, universities and not-for-profit laboratories throughout Brazil. Dr Erbisch worked with representatives of the Brazilian government, several universities and a major research foundation to provide instruction related to intellectual property management.

**Other Linkages**

In March 2000, Dr. Catherine Ives attended the Biotechnology Industry Organization (BIO) annual meeting in Boston, MA. Dr. Ives established a number of industry contacts at the meeting and attended a number of sessions including *The Consumer Market for Value-Added Biotech Foods*, and *the Past, Present and Future of Biotechnology Economics*.

Dr. Catherine Ives served as a member of the Bean/Cowpea CRSP Search Committee formed to select an Assistant Director for the B/C CRSP.

Dr. Ives attended a management course from September 25-29, 2000, entitled *Managing Innovation in Agribusiness*, sponsored by Monsanto and conducted by the Kenan-Flagler Business School at the University of North Carolina at Chapel Hill.

Dr. Ives and other Institute members held a meeting in March with Dr. Henk Knipscheer, Senior Managing Director, Global Operations, Winrock International to discuss the possibility of collaborating on the delivery and dissemination of biotechnology-derived crops. While having little experience in the development of transgenic crops, Winrock does have extensive experience in the delivery and

dissemination of technology. ABSP will continue to engage NGOs in discussions of how to effectively deliver agricultural biotechnology.

## Publications

- K. M. Maredia and C.L. Ives. 2000. Capacity building in Biosafety: Experience of the USAID – Agricultural Biotechnology Support Project. Proceedings of the International Workshop on Biosafety and Food Safety of Genetically Engineered Products, Jakarta, Indonesia, February 1 – 2, 2000.
- K. M. Maredia, F. H. Erbisch and M. J. Sampaio. 2000. Technology Transfer Offices for Developing Countries. *Biotechnology and Development Monitor*, The Netherlands, No. 43 (September 2000), pages 15 - 18.
- Grumet R, Kabelka E, McQueen S, Wai T, Humphrey R. 2000. Characterization of sources of resistance to the watermelon strain of papaya ringspot virus in cucumber: allelism and co-segregation with other potyvirus resistances. *Theor. Appl. Genet.* 101:463-472.
- Papadopoulou E, Grumet R. Transformation of melon (*Cucumis melo* L.). In *The Handbook of Transgenic Food Plants*. Hui YH (ed). Marcel Decker Inc. In press.
- Lecoq H, Dafalla G, Desbiez C, Lip C, Delecalle B, Lanina T, Ullah Z, Grumet R. Biological and molecular characterization of Moroccan watermelon mosaic virus and a related Potyvirus isolate from Eastern Sudan. Submitted to *Plant Disease*.
- S.M.Khalil, M.A.Badawi, Kh.A.Abdel-Ati and M.A.Madkour (2000). Plant regeneration via somatic embryogenesis from cotyledons in cucumber (*Cucumis sativus* L. cv. Beit Alpha). *Arab Journal of Biotechnology* 3(1):87-96.
- Pett, W., D. Douches and E. Grafius. 2000. Insect control: Durability and breakdown of resistance. In: *Proceedings of the International Workshop on Transgenic Potatoes for the Benefit of Resource-Poor Farmers in Developing Countries*. Manchester, United Kingdom. pg. 82-86.
- Bulla, L. et al. Expression in *Spodoptera frugiperda* (Sf21) Insect Cells of BT-R<sub>1</sub>, a Cadherin-related Receptor from *Manduca sexta* for *Bacillus thuringiensis* Cry1Ab Toxin. Accepted for publication in *Protein Expression and Purification*.
- Bulla, L. et al. Developmental and Tissue Specific Expression of BT-R<sub>1</sub> in *Manduca sexta*. Submitted to *Journal of Experimental Biology*.
- Bulla, L. et al. Insect Resistance to *Bacillus thuringiensis* Insecticidal Toxins. Submitted to *Journal of Insect Biochemistry and Molecular Biology*.
- Mohammed A, Douches D, Pett W, Grafius E, Coombs J, Li L and Madkour M. 2000. Evaluation of potato tuber moth (Lepidoptera:Gelechiidae) resistance in tubers of *Bt-cry5* transgenic potato lines. *Journal of Economic Entomology* 93(2): 472–476.
- Bahieldin, A.; R. Qu, W.E. Dyer, A.S. Haider and M. Madkour (2000). A modified procedure for rapid recovery of transgenic wheat plants. *Egyptian J. Genetics and Cytology*, 29: 11-23.

## ABSP Travel January – December 2000

Month	Traveler	Institution	Country	Purpose of travel	Destination
January					
	Dr. Catherine Ives	Michigan State University	USA	Meet w/J. Lewis, USAID	Washington, DC
	Ms. Melba Lacey	Michigan State University	USA	Egypt Symposium Pre-Planning	Cairo, Egypt
	Dr. Catherine Ives	Michigan State University	USA	Egypt Symposium Pre-Planning	Cairo, Egypt
	Dr. Karim Maredia	Michigan State University	USA	Biosafety Workshop & visit KIAT	Jakarta & Bogor, Indonesia
February					
	Dr. Karim Maredia	Michigan State University	USA	AUTM 2000 Annual Meeting	Atlanta, GA
March					
	Dr. Catherine Ives	Michigan State University	USA	UC-Davis meeting on Vit. A research subcontract	UC-Davis, CA
	Dr. Catherine Ives	Michigan State University	USA	BIO 2000 Conference & subcontractor visits	Boston, MA, TX, CA
	Dr. Magdy Madkour	AGERI	EGYPT	BIO 2000 Conference & subcontractor visits	Boston, MA, TX, CA
	Dr. Hanaiya El-Itriby	AGERI	EGYPT	Bio 2000 Conference	Boston, MA
	Dr Andrea Johanson	Michigan State University	USA	Biosafety Workshop 2/CABI UK	Florence, Italy/UK
	Dr. Mwananyanda Lewanika	National Institute for Science & Industry Research	ZAMBIA	Biosafety 2: Advanced Reseach & Procedures	Florence, Italy
	Dr. Hisham El-Sheshtawy	AGERI	EGYPT	Biosafety 1: Science & Policy in Risk Assessment..	Trieste, Italy
April					
	Dr. Patricia Traynor	VA Polytechnic Institute & State University	USA	Meet w/ABSP staff & researchers & Hort/CSS seminar	East Lansing, MI
	Dr. Karim Maredia	Michigan State University	USA	Egypt Symposium 2000	Cairo, Egypt
May					
	Dr. Donald Plucknett	Agricultural Research & Development International	USA	Egypt Symposium 2000	Cairo, Egypt
	Dr. Maria Jose Sampaio	EMBRAPA	BRAZIL	Egypt Symposium 2000	Cairo, Egypt
	Dr. David Morton	American Ag-Tec International, Ltd.	USA	Egypt Symposium 2000	Cairo, Egypt
	Dr. Desh Pal Verma	Ohio State University	USA	Egypt Symposium 2000	Cairo, Egypt
	Dr. Russel Freed	Michigan State University	USA	Egypt Symposium 2000	Cairo, Egypt
	Mrs. Susan Gibbons	Michigan State University	USA	Egypt Symposium 2000	Cairo, Egypt
	Dr. Ann Ferguson	ZENECA Plant Science	UNITED KINGDOM	Egypt Symposium 2000	Cairo, Egypt
	Mr. Mohamed Saleh Tawfik	Michigan State University	USA	Egypt Symposium 2000	Cairo, Egypt
	Mr. Hussein Soliman	US Grains Council	EGYPT	Egypt Symposium 2000	Cairo, Egypt

Month	Traveler	Institution	Country	Purpose of travel	Destination
	Dr. Josette Lewis	USAID/G/EGAD	USA	Egypt Symposium 2000	Cairo, Egypt
	Dr. Taylor Johnston	Michigan State University	USA	Egypt Symposium 2000	Cairo, Egypt
	Dr. David Douches	Michigan State University	USA	Egypt Symposium 2000	Cairo, Egypt
	Dr. Michael Schechtman	USDA	USA	Egypt Symposium 2000	Cairo, Egypt
	Dr. Wayne Loescher	Michigan State University	USA	Egypt Symposium 2000	Cairo, Egypt
	Ms. Melba Lacey	Michigan State University	USA	Egypt Symposium 2000	Cairo, Egypt
	Dr. Terry Meyer	Pioneer Hi-Bred International, Inc.	USA	Egypt Symposium 2000	Cairo, Egypt
	Dr. Lee Bulla	University of Texas Southwestern Medical Center	USA	Egypt Symposium 2000	Cairo, Egypt
	Dr. Luis Destefano-Beltran	Demegen, Inc. & NovaTero Foundation	USA	Egypt Symposium 2000	Cairo, Egypt
	Dr. Catherine Ives	Michigan State University	USA	Egypt Symposium 2000	Cairo, Egypt
	Dr. Molly Jahn	Cornell University	USA	Egypt Symposium 2000	Cairo, Egypt
	Dr. Patricia Traynor	VA Polytechnic Institute & State University	USA	Egypt Symposium 2000	Cairo, Egypt
	Dr. Walter Pett	Michigan State University	USA	Egypt Symposium 2000	Cairo, Egypt
	Dr. George Moriarty	Cornell University	USA	Egypt Symposium 2000	Cairo, Egypt
	Dr. Richard Sawyer	Private Consultant	USA	Egypt Symposium 2000	Cairo, Egypt
	Dr. Ed Grafius	Michigan State University	USA	Egypt Symposium 2000	Cairo, Egypt
	Dr. John Jantz	Cornell University	USA	Egypt Symposium 2000	Cairo, Egypt
	Dr. Peter Matlon	United Nations Development Program (UNDP)	USA	Egypt Symposium 2000	Cairo, Egypt
	Dr. Eva-Maria Scholz-Tonga	Aventis	GERMANY	Egypt Symposium 2000	Cairo, Egypt
	Dr. Mark Condon	American Seed Trade Association	USA	Egypt Symposium 2000	Cairo, Egypt
June					
	Dr. Walter Pett	Michigan State University	USA	Potato Meeting in UK	Manchester, UK
July					
	Dr. Bambang Dwiymoko	Ministry of Health	INDONESIA	International Food Safety course	MSU, East Lansing, MI
	Dr. Johan Brink	Agricultural Research Council	SOUTH AFRICA	6th Biosafety Symposium/Canada, visit MSU/return	Saskatoon, Canada & E. Lansing
	Dr. Ir Basuki	Biotechnology Research Unit for Estate Crops	INDONESIA	Interantional Food Safety Course	MSU, East Lansing, MI
	Mr. Motaz Moniem	AGERI	EGYPT	MSU IPR Internship Program	East Lansing, MI
	Mr Tanton Subagyo	Intellectual Property & Transfer Technology Office	INDONESIA	MSU IPR Internship	East Lansing, MI
	Mr. Walid Maaty	AGERI	EGYPT	Research at UT-Dallas w/Lee Bulla	UT-Dallas, Texas
	Mr. Badaweysa Saad Hamza	Agricultural Research Center (ARC)	EGYPT	MSU Food Safety Short Course	East Lansing, MI

Month	Traveler	Institution	Country	Purpose of travel	Destination
<b>August</b>					
	Ms. Pat Sterns	Institut National de la Recherche Agronomique	FRANCE	Grades & Standards assessment	Kenya
	Dr Jordan Tatter	Hanson Cold Storage Company	USA	Grades & Standards Assessment	Kenya (based in Nairobi)
	Dr. Vince Hegarty	Michigan State University	USA	Grades & Standards assessment	Kenya
	Dr. Mylene Kherallah	IFPRI	USA	Grades & Standards assessment	Kenya
	Dr. Craig Harris	Michigan State University	USA	Grades & Standards assessment	Kenya
	Ms. Jane Mumbi Ngige	Biosystems ,Research & Develipment	KENYA	Grades & Standards assessment	Kenya
	Dr. Karim Maredia	Michigan State University	USA	Cucurbits Group Annual Meeting	Cornell Univ., Ithaca, NY
<b>September</b>					
	Dr. Johan Brink	Agricultural Research Council	SOUTH AFRICA	Meet for proposed activities/Southern Africa	Gabarone, Botswana
	Dr. Karim Maredia	Michigan State University	USA	South Africa technical site visits	South Africa
	Dr. Walter Pett	Michigan State University	USA	South Africa technical site visits	South Africa
<b>October</b>					
	Dr. Catherine Ives	Michigan State University	USA	Intl Food & Nutrition Conf./Biotechnolgy Session	Tuskegee, Alabama
	Ms. Pat Sterns	Institut National de la Recherche Agronomique	FRANCE	Grades & Standards assessment in Malawi	various sites in Malawi
	Dr. Catherine Ives	Michigan State University	USA	Food Prize/Bioethics Meet/IA& Haas Bus. Sch.,CA	Des Moines, IA & Berkeley, CA
	Dr. David Toomey	World Link Associates	USA	Grades & standards assessment in Malawi	various sites in Malawi
	Dr. Karim Maredia	Michigan State University	USA	CGIAR Center's Week Conference	Washington, DC
	Ms. Melba Lacey	Michigan State University	USA	Meet w/Monsanto to discuss subcontract	St. Louis, MO
	Dr. Muhammad Herman	CRIFC	INDONESIA	Visit ABSP faculty & staff at MSU	East Lansing, MI
<b>November</b>					
	Dr. Catherine Ives	Michigan State University	USA	BioEarn meet, Wafula meet., Reg. biosafety meet.	Kenya & South Africa
	Mr. Charles Jumbe	Bunda College of Agriculture	MALAWI	Grades & Standards de-brief meeting for Malawi	Johannesburg, South Africa
	Dr. Lawrence Busch	Michigan State University	USA	Present results-grades & standard assessment/Kenya	Nairobi, Kenya
	Dr. Catherine Ives	Michigan State University	USA	Global Seminar & International Learning Workshop	Cornell Univ., Ithaca, NY

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

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## Research Team

Rebecca Grumet, Michigan State University  
Molly Jahn, Cornell University

Dr. Atek Sadik, AGERI, Egypt  
Dr. Hamdy El-Doweny, Horticultural Research Inst., Egypt

## Overall project goal

Our project 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 (R. Grumet) and couple the transformation capacity with ongoing breeding efforts (M. Jahn) to develop high quality cucurbits with multiple virus and disease resistances.

## Importance of the problem

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 several viruses including the potyviruses, zucchini yellow mosaic virus (ZYMV), watermelon mosaic virus (WMV), the watermelon strain of papaya ringspot virus (PRSV-W), and the cucumovirus, cucumber mosaic virus (CMV). 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 in the U.S. A major limitation to more widespread application of this technology to various cucurbit crops is the 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 major objective of this project is to develop a novel, non-regeneration based system for cucurbit transformation. To this end we are using two approaches. In the first approach we are working to adapt the electrotransformation system that was recently developed by Dr. Richard Allison (Michigan State University) for use with legume crops. Both soybean and cowpea have been transformed using this method: approximately 10% of the treated plants subsequently produce transgenic progeny. If successful, this methodology would have value for any future traits to be incorporated; would have the added benefit of being broadly applicable across genotypes and even species, should be readily replicated in other laboratories, and would avoid the time, effort, expense and sophistication necessary for regeneration based systems. These features should make transformation technology more readily transferable to developing countries. In the second approach we are working to adapt a pollen-tube transformation method that has been widely used in China for several species including wheat, cotton, soybean, rice, (Zeng et al. 1994; Huang et al. 1999; Hu and Wang, 1999; Xie and Fan, 1992; Xu et al. 1991) and recently watermelon (Chen et al. 1999), but has received little attention in Western laboratories. The principle of this method is to apply DNA at a time when it can traverse through the pollen tube and gain access to the fusing pollen and egg cells. The egg or zygote at this early developmental stage is considered a protoplast, and so presumably allows easy entry of exogenous DNA (Chong et al. 1998). If successful, this method would be even simpler, and involve less sophisticated equipment than electrotransformation.

Because of interest expressed by collaborating ABSP countries, Egypt and Indonesia, we have initiated our efforts with cucumber. We also have been successful in establishing *Agrobacterium*-mediated transformation of cucumber.

## 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 produced transgenic squash lines showing resistance to ZYMV in the greenhouse and field.

In the current phase of the project our attention is focused primarily on the development of a non-regeneration dependent transformation system as described above. Our initial goals were to determine the parameters appropriate for electrotransformation of cucumber, including appropriate stage of seedling development, handling procedures before, during, and after

electrotreatment to ensure transformation and recovery, and the appropriate electrotransformation settings for cucumber. Using ethidium bromide stained DNA to monitor entry of DNA into the cucumber tissue we were successful in modifying the original soybean-based procedure to adapt the DNA delivery system. We obtain 90 - 100% survival of the electrotreated plants.

Once these conditions were established, we treated approximately 200 plants and transferred them to the greenhouse for growth and fruit production. Cucumber plants subjected to the transformation procedure did not exhibit any apparent damage as compared to the non-treated control plants. When vegetative tissue of the treated plants was sampled by PCR analysis, 23 individuals (ca. 10%), indicated a positive response for incorporation of the introduced marker gene. This suggested stable integration of the gene that could be observed in upper leaves formed weeks after the initial treatment. No amplification product was observed in samples derived from control, non-treated plants. This frequency is very promising and is consistent with the results that have been obtained from soybean.

The electrotreated plants were hand pollinated to produce seed in the greenhouse. Fruit were harvested from approximately 150 plants. Seed were extracted from the harvested fruit and screened for transfer of the introduced gene to the next generation. Results of screening seeds from ca. 80 fruit did not yield PCR-positive individuals.

One possible explanation for the discrepancy between results from the originally treated plants and their progeny is that the original treated plants are chimeric, i.e., they contain a mixture of transformed and non-transformed tissue. Given the nature of the original electrotreatment, it is reasonable to assume that only portions of the plant, and not the whole plant will become transformed. Since in the greenhouse we are only able to set 1-3 fruits per plant, when producing progeny we are not able to sample the whole plant. This can be contrasted with the situation with soybean where small numbers of seeds are set throughout the whole plant, rather than large numbers localized in one or a few fruits. This is a potential problem for cucurbits that we anticipated from the outset, and so we considered the possibility of modifying the approach if the initial method did not yield transgenic progeny. Modifications tested included introduction of a selection step to reduce chimerism of regenerated plants, and electrotransformation of floral meristems as described below.

At the same time that we were working to develop an electrotransformation system for cucumber and other cucurbit species, we began to revisit *Agrobacterium* based systems for cucumber, and have had very encouraging results. Cucumber transformation has been very recalcitrant; in the published transformation reports, regeneration had been via somatic embryogenesis (Chee, 1990; Hammar and Grumet, 1990; Chee and Slightom, 1992; Sarmiento et al. 1992; Schultz et al. 1995; Nishibashi et al. 1996; Rajharo et al. 1996). These methods were limited by low efficiency, extended time in tissue culture, and lack of reproducibility among laboratories.

Last summer we tested a new method developed by Tabei et al. (1998) for a Japanese genotype and have been able to obtain reproducibly high regeneration rates with the American pickling cucumber, Straight 8. This system has the advantage of regeneration via organogenesis, which like the methods for melon, is more efficient and requires less time in tissue culture. The time in tissue culture to produce visible shoots is approximately 3 weeks, and by 4-5 weeks shoots can be transferred to elongation medium. We made modifications in the protocol to reduce vitrification of the regenerated shoots. Our next steps were to utilize this regeneration system for transformation and to test its usefulness with other cucumber genotypes as described below.

## Research progress

### ***Electrotransformation.***

At the time that we were performing these experiments, further analysis of the soybean system indicated that when transgenic progeny were obtained, in each case it was from the first pod to set seed (Allison et al., unpublished). This led to a revised view of the electrotransformation procedure suggesting that the gene introduction that leads to successful production of transgenic progeny may be directly incorporated into the developing floral primordium that is present at the time of electrotransformation. Given this information, we revised the treatment protocols to treat older seedlings (7-10 days) at a time when they are in the process of initiating floral primordia (Goffinet, 1990), and switched to the use of the gynococious pickling cucumber breeding line GY14, which would allow for fruit set at the earliest flowering nodes. Flowers were pollinated at the first four flowering nodes. Fruit have been collected from approximately 200 plants for subsequent screening of seeds.

### ***Pollen tube-mediated transformation***

Two similar techniques are used. The first involves cutting the stigma of the flower following pollination, then placing a droplet of DNA on the cut surface of the style (Zeng et al. 1994; Hu and Wang, 1999). In the second, exogenous DNA is injected directly into the ovary following pollination (Hu and Wang, 1999; Chen et al. 1999). The size of the ovary of the cucumber should make it a good candidate for these approaches. Efforts were initiated with the gynococious line GY14 to obtain earlier fruit set. Since appropriate timing between pollination and treatment with DNA is necessary to enable delivery to the fertilized egg cell, treatments were performed at 24 and 48 hours post-pollination using both excision of the stigma and injection into the ovary. Both cut and uncut plasmid DNA are also being tested. Approximately 225 fruits were produced. Seedlings from these fruit are being screened for expression of the introduced *Bar* gene by exposing excised leaf punches to the herbicide Basta (glufosinate). Individuals showing reproducible promising results will be verified by PCR or Southern analyses.

### ***Agrobacterium-mediated transformation***

We have used the *Agrobacterium*-mediated transformation system to successfully transform the monoecious and gynoeious cucumber genotypes, Straight 8 and GY14. At least five gene constructs, including the Arabidopsis *CBF* (C-repeat binding factor) gene (Thomashow, 1999) being used by Mohamed Tawfik, an Egyptian Ph.D. student from AGERI, to engineer increased environmental stress tolerance, have been successfully introduced as verified by PCR analysis. In addition, for several of the *CBF* transgenics, fruits were produced in the greenhouse, and gene transfer to the progeny was verified by PCR and northern blot analysis. Northern analysis of several transgenic individuals showed a range of expression of the *CaMV 35S-CBF1* and *-CBF-3* genes. Initial testing of segregation ratios for three families suggests single gene integration events.

We also tested the Indonesian cultivar Hijau Raket, but it did not regenerate well in this system. Although requested, we were not able to obtain Egyptian seed for testing in this system. Overall, we can conclude that we have an effective transformation system for cucumber suitable for the American genotypes tested.

## **Publications**

**Grumet R**, Kabelka E, McQueen S, Wai T, Humphrey R. 2000. Characterization of sources of resistance to the watermelon strain of papaya ringspot virus in cucumber: allelism and co-segregation with other potyvirus resistances. *Theor. Appl. Genet.* 101:463-472.

Papadopoulou E, **Grumet R**. Transformation of melon (*Cucumis melo* L.). *In* The Handbook of Transgenic Food Plants. Hui YH (ed). Marcel Decker Inc. In press.

Lecoq H, Dafalla G, Desbiez C, Lip c, Delecolle B, Lanina T, Ullah Z, **Grumet R**. Biological and molecular characterization of Moroccan watermelon mosaic virus and a related Potyvirus isolate from Eastern Sudan. Submitted to Plant Disease.

## **Literature Cited**

Chee PP. 1990. Transformation of *Cucumis sativus* tissue by *Agrobacterium tumefaciens* and the regeneration of transformed plants. *Plant Cell Rep.* 9:245-248.

Chee PP, Slightom JL. 1992. Transformation of cucumber tissues by microprojectile bombardment: identification of plants containing functional and non-functional transferred genes. *Gene* 118:255-260.

Chen WS, Chiu CC, Liu HY, Lee TL, Cheng JT, Lin CC, Wu YJ, Chang HY. 1999. Gene transfer via pollen-tube pathway for anti-fusarium wilt in watermelon. *Biochem. Molec. Biol. Internat.* 49:1201-1209.

- Chong K, Bao S, Xu T, Tan K, Liang T, Zeng J, Huang H, Xu J, Xu Z. 1998. Functional analysis of the *ver* gene using antisense transgenic wheat. *Physiologia Plantarum* 102:87-92.
- Fang G, Grumet R. 1993. Genetic engineering of potyvirus resistance using constructs derived from the zucchini yellow mosaic virus coat protein gene. *Mol. Plant Mic. Interact.* 6:358-367.
- Goffinet MC. 1990. Comparative ontogeny of male and female flowers in *Cucumis sativus*. In: *Biology and Utilization of the Cucurbitaceae* (eds. DM Bates, RW Robinson, C Jeffrey). Cornell Univ. Press, NY p. 288-304.
- Grumet R, Yadav RC, Akula G, Hammar S, Provvidenti R. 1995. Genetic engineering of virus resistance in cucurbit crops. *Proceedings Cucurbitaceae '94*. In: *Evaluation and enhancement of cucurbit germplasm*. Lester GE, Dunlap JR (eds). P. 17-22.
- Hammar S, Grumet R. 1990. Regeneration and *Agrobacterium* mediated transformation of cucumber. *HortScience* 25:1070.
- Hu CH, Wang L. 1999. In plant soybean transformation technologies developed in China: procedure, confirmation, and field performance. *In Vitro Cell. Dev. Biol.* 35:417-420.
- Huan G, Dong Y, Sun J. 1999. Introduction of exogenous DNA into cotton via the pollen-tube pathway with GFP as a reporter. *Chinese Sci. Bull.* 44:689-701.
- Nishibayashi S, Hayakawa T, Nakajima T, Suzuki M, Kancko H. 1996. CMV protection in transgenic cucumber plants with an introduced CMV-O CP gene. *Theor. Appl. Genet.* 93:672-678.
- Raharjo SHT, Hernandez MO, Zhang YY, Punja ZK. 1996. Transformation of pickling cucumber with chitinase encoding genes using *Agrobacterium tumefaciens*. *Plant Cell Rep.* 15:591-596.
- Sarmiento GG, Alpert K, Tang FA, Punja ZK. 1992. Factors influencing *Agrobacterium tumefaciens* mediated transformation and expression of kanamycin resistance in pickling cucumber. *Plant Cell Tiss Org Cult.* 31:185-193.
- Tabei Y, Kitade S, Nishizawa Y, Kikuchi N, Kayano T, Hibi T, Akutsu K. 1998. Transgenic cucumber plants harboring a rice chitinase gene exhibit enhanced resistance to gray mold (*Botrytis cinerea*). *Plant Cell Rep.* 17:159-164.
- Thomashow MF. 1999. Plant cold acclimation: freezing tolerance genes and regulatory mechanisms. *Annu. Rev. Plant Phys. Mol. Biol.* 50:571-599.
- Xie DX, Fan YL. 1992. Transgenic rice plant of a superior Chinese cultivar Zhonghua No. 11 containing the Bt endotoxin gene in its genome. *Science in China (Ser.B)* 35:566-569.
- 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

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## Research Team

### **Cornell University**

Dr. Molly Jahn

Mr. George Moriarty

Mr. Mark Henning

## Research progress

### **Overview**

This year we continued to run large greenhouse and field-based screens of advanced cucurbit 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 15 seed companies from around the world, and we have sent seed from this program to Africa, Asia and Latin America for trials. 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. We also contributed to the symposium held in May, 2000 in Cairo and have identified private sector cooperators who are conducting major trials of ABSP germplasm in South Africa, Indonesia, and Brazil. Major trials of this material have also been or are currently being conducted in Jordan and the Philippines.

### **Multi-virus resistance Melons**

#### ***Orange soft flesh types***

This summer we planted 8 Topmark F<sub>5</sub> lines that had been screened for Cucumber Mosaic Virus (CMV), Papaya Ringspot Virus (PRV), Watermelon Mosaic virus (WMV) and Zucchini Yellows Mosaic virus (ZYMV) in a winter greenhouse generation harvested in May, 2000. The winter Topmark generation segregated for resistance to PRV, WMV and ZYMV. All lines were susceptible to CMV. In our summer planting, the eight F<sub>5</sub> lines derived from the winter test were screened separately for PRV, WMV, and ZYMV. Our summer results show that we have material that is resistant/tolerant to PRV, WMV and ZYMV, with particularly high levels of resistance/tolerance to WMV. Plants inoculated with WMV had no symptoms or very mild symptoms even after fruit set. We do not yet have all three resistances combined.

In the TamUvalde background we planted 5 F<sub>10</sub> lines and one F<sub>6</sub> line that had been screened for CMV, PRV, WMV and ZYMV in a winter greenhouse generation harvested in May 2000. The winter generation segregated for resistance to PRV, WMV and ZYMV. In our summer planting, these 6 lines were screened with WMV and ZYMV. We observed resistance/tolerance to WMV. Some lines were segregating for ZYMV.

In our Cornell 339 inbred lines we have made considerable progress in stabilizing resistance to CMV, PRSV, WMV, and ZYMV. This summer we planted 40 lines of varying generations. These lines were screened in the field for single or multiple resistances to CMV, PRV, WMV and ZYMV. Our field trial this year gave good evidence for successfully bringing in additional CMV and WMV resistance to our 339 lines. Much of this material has PMR (powdery mildew resistance), (race 1 and possibly race 2).

### ***Green/White crisp flesh types***

In the honeydews we planted 46 F<sub>6</sub> and F<sub>7</sub> lines that had been screened for CMV, PRV, WMV and ZYMV in a winter greenhouse generation harvested in May, 2000. The winter generation segregated for resistance to PRV, WMV and ZYMV. We had very little resistance to CMV. In our summer planting, the 46 lines derived from the winter test were screened for anywhere from 1 to 3 viruses. Our summer results show that we have some lines with single virus resistance and some with two resistances. Most of these lines also have PMR with limited or almost no autogenic necrosis.

The multi-virus melon breeding program has been transitional during 2000 in that we are trying to finish off the types we have been working with and update our recurrent parents, adding Galia, Ananas and other types of more importance in tropical areas. This winter, we will cross the leading single and multi-resistant breeding lines with new recurrent parents with the hope of taking F<sub>2</sub> seed to the field for disease screening. We will also progeny test lines that looked good in the field for resistance/tolerance to the four viruses and intercross best plants. In the western shipper class, we are using Oro Rico and plan to bring in Fusarium race 2. We are also using updated Honeydew and Eastern cantaloupe parents.

In September 2000, we chose 12 lines out of our summer 2000 field planting to send to three trial locations. We based our selections on virus resistance and how close the resistance was to breeding true. In addition, we selected for powdery mildew resistance (PMR), although never at the expense of virus resistance. While the melon types represented in these 12 lines will not be appropriate for the range of markets in each country, we want to see how they perform under standard growing conditions in each location and how they hold up to disease pressure. This will help us evaluate the effectiveness of our resistance. We are currently conducting a large winter greenhouse virus screen (CMV, PRSV, WMV, and ZYMV) on lines harvested and selected for virus resistance from the summer 2000 field planting. Best lines will be selected, self-pollinated and crossed to the new recurrent parents.

### **Multiple virus & disease resistance in *Cucurbita pepo* Eskanderany**

Last winter all of the selected material from this program was tested in the greenhouse for all 4 viruses separately and every single progeny was found to be still segregating for at least one if not more virus resistances. Self-pollinations and crosses were made to pyramid resistances and progenies were brought to the field during the summer, 2000. Field-testing this year has shown higher levels of resistance to individual viruses but we still haven't succeeded in creating lines homozygous to all four viruses. We also planted a screen of varieties called Eskandarany and have found a very broad range of fruit type, especially major differences in fruit color that range from white to green. As part of this effort we have located a better recurrent parent and have incorporated that into this ongoing project.

We have sent 5 of our best Eskandarany lines to the three international trial locations. Selections were based on virus resistance and fruit type. We are currently starting to move our virus resistance into new commercial types. This is being done in collaboration with one U.S. seed company that has provided the new recurrent parents. Some of these commercial types may be more acceptable in markets in the three trial countries.

For summer 2000 we screened 84 breeding lines with 4 different viruses (CMV, PRSV, WMV, and ZYMV). The survivors are currently being screened in the greenhouse during the winter 2000-2001 for CMV, PRSV, WMV, and ZYMV. Best lines will be selected, self-pollinated and crossed to the new recurrent parents.

### **Multiple Disease Resistance in Cucumber**

#### ***Fine-spine, glossy Poinsetts (Beit Alpha type)***

We are currently breeding this type for resistance to 10 diseases, the four viruses, two mildews, scab, and three leafspot organisms. We sent out 5 of our best lines to the four locations. Selections were based on disease resistance and fruit type. We are aiming for a longer fruit type with smooth skin and few spines. Lastly, it appears we have some parthenocarpic (seedless fruit production) tendency in some of our lines. This trait could be valuable for the greenhouse grown cucumber market.

Below is a photo of one of our Beit Alpha breeding lines:



Some of our Beit Alpha lines have been sent this winter to Seminis for screening for Target Leafspot (TLS). Also, we will be conducting a virus (CMV, PRSV, WMV, and ZYMV) test on Beit Alpha lines in the near future.

Lastly, we have licensed Marketmore 97 (North American slicer type) to a seed company in Pakistan for production and sale.

### ***ABSP Sponsored International Cucurbit Trials***

This year, in cooperation with private seed companies, we are trialing our leading virus + powdery mildew resistant cucurbit lines in three locations: Indonesia (P.T. East West Seed Indonesia), South Africa (Alpha Seed), and Brazil (Agroflora/Sakata). We have existing relationships with East West Seed and Agroflora/Sakata, while we are just beginning to cooperate with Hytech and Alpha Seed. This spring we will be visiting these three locations to evaluate our material. We also have a major trial planted south of Amman Jordan by Syngenta and recently received extensive evaluation data from East West Seed Co. in the Philippines

We have sent 5 of our best gummy stem blight lines to the three international trial locations. Selections were based on fruit type and gummy stem blight resistance. In the near future we will be conducting a gummy stem blight test on lines harvested from the summer 2000 field planting. We will self the most resistant lines, which will be planted this summer for further evaluation for type and resistance. We also will incorporate our new recurrent (commercial) parents.

## International Collaboration

We have sent 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
Disease resistant <i>C. moschata</i>	New Zealand
Disease resistant <i>C. moschata</i> , multiple virus resistant Eskandarany, multiple virus and disease resistant melon, multiple virus and disease resistant Beit Alpha cucumber	South Africa (2 cooperators) Brazil
Multiple virus and disease resistant melon, multiple virus and disease resistant cucumbers: Beit Alpha & North American slicer types	Indonesia
Eskandarany, multiple virus and disease resistant melon, multiple virus and disease resistant Beit Alpha cucumber	Egypt
Multiple virus resistant <i>C. moschata</i>	Philippines
Marketmore 97	Pakistan

There area also a number of U.S. and European seed companies that are actively breeding with the above material in various locations around the world including Latin America, Mexico, France, the Netherlands, Turkey and India. Syngenta is conducting the most extensive of these trials this spring in Jordan.

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

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## Research Team

### AGERI

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Gihan M. Zeinhom

## Project partners

Dr. Rebecca Grumet, Michigan State University.

Dr. Molly Kyle Jahn, Cornell University.

## 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

Cucurbit species include a variety of high value crops (e.g., cucumber, melon, watermelon and squash) that play important roles in local diets and as export crops. In Egypt, the cultivated cucurbits are infected with some common viruses, i.e., ZYMV, CMV and WMV, causing reduction in yield and quality. The control of such viruses using insecticides and/or inspection

and rouging is ineffective. Therefore, cultivars resistant to such viruses would be the most effective means for its control.

## Project background

Plant viruses can cause devastating losses to many agricultural crops by reducing either the yield or the quality of the crop. Control of these virus diseases through use of chemicals, inspection and rouging was not effective. Possible options to control plant viruses should include developing resistant transgenic plants *via* a genetic engineering approach by introducing the viral coat protein gene (Clough and Hamm, 1995). Coat protein-mediated protection (CPMP) (Gonsalves and Slightom, 1993; Fitch and Beachy, 1993 and Lomonosoff, 1995) constitutes a major breakthrough to facilitate the incorporation of virus resistance in crop plants.

In Egypt, the squash-cultivated area was reported to be affected by ZYMV. Providenti *et al.* (1984) reported that ZYMV was the most serious virus affecting squash, muskmelon (*Cucumis melon* L.) and other cucurbits. The control of ZYMV based on using insecticides and/or inspection and rouging (Sherf and MacNab, 1986) was ineffective, but some limited success has been obtained with the use of mineral oil sprays (Webb and Linda, 1993). Khalil *et al.* (1999) produced transgenic squash plants resistant to ZYMV-CT strain under the ABSP/AGERI project.

## Rational for approach

Cucurbit species include a variety of high value crops (e.g., cucumber, melon, watermelon, squashes) that play important roles in local diets and as export crops. The area under cultivation with squash crop in Egypt is around 78,000 feddans and produces about 568,000 tons. In addition, the export values for melon and watermelon exceed \$1 million annually (\$749,000 and \$310,000, respectively). In Egypt, many diseases, causing reduction in yield and quality, infect cultivated cucurbits. These crops can be completely destroyed when infected by zucchini yellow mosaic potyvirus (ZYMV), cucumber mosaic cucumovirus (CMV) and/or watermelon mosaic potyvirus (WMV). The control of such viruses based on using insecticides and/or inspection and rouging was ineffective. Transgenic varieties that are virus resistant, based on virus-derived transgenes have been widely demonstrated to be an effective strategy for control of such viruses and could increase productivity and reduce inputs.

## Previous research

1. At AGERI, we were successful in introducing the 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 *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 trails at Sids Experimental Station for three seasons.
  - c. 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. Evaluation of highly ZYMV-tolerant squash lines under open field conditions.
  3. Establishment of regeneration system in cucumber.
  4. Establishment of transformation system in cucumber.
  5. Evaluation of Ro-transformed cucumber plants.

## Specific project objectives

- ❑ Evaluation of the multiple virus resistant squash, melon and cucumber lines obtained from Cornell University under field conditions.
- ❑ Continue the evaluation of highly ZYMV-tolerant squash lines containing the cp-gene of ZYMV-CT strain (R5) under open field conditions (in different locations in Egypt).
- ❑ Re-evaluation of highly ZYMV-tolerant melon lines containing the cp-gene of ZYMV-CT strain (R3) under greenhouse and/or open field conditions (in different locations in Egypt).
- ❑ Isolation, identification and purification of watermelon mosaic potyvirus (WMV).
- ❑ Isolation, cloning and sequencing the coat protein gene of WMV for construct preparation.
- ❑ Establishment of regeneration system in watermelon.
- ❑ Establishment of transformation system in watermelon.

## Research progress

### **Objective 1: Evaluation of the multiple virus resistant squash, melon and cucumber lines obtained from Cornell University under field conditions**

A number of 30 lines consisting of squash, cucumber and cantaloupe were sent to AGERI by Dr. M. Kyle (Cornell University) for evaluation of their virus resistance under field conditions at Sids Experimental Station, Beni Suef Governorate, Egypt. These lines were left to natural infection with virus(s) and artificial inoculation was not carried out. The evaluation was based on the development of virus-like symptoms on these plants. Results showed that approximately

90% of the plants showed no symptoms after 12 week from planting. Results are represented in Figures 1-6.



**Figure 1.** Evaluation of multiple virus resistant squash (Top), melon (Middle) and cucumber (Bottom) lines from Cornell University under field conditions at Sids Experimental Station, Beni Suef, Egypt.



**Figure 2.** Two different squash lines from Cornell University showing severe (Top) and mild (Bottom) virus-like symptoms 12 weeks post planting under field conditions at Sids Experimental Station, Beni Suef, Egypt.



**Figure 3.** Squash fruits belonging to lines 99-101-1 L0 (1) and 99-104-1 L0 (3) from Cornell University evaluated under field conditions at Sids Experimental Station, Beni Suef, Egypt.



**Figure 4.** Seed production of two resistant cucumber lines from Cornell University evaluated under field conditions at Sids Experimental Station, Beni Suef, Egypt.



**Figure 5.** Two different melon lines from Cornell University evaluated under field conditions at Sids Experimental Station, Beni Suef, Egypt showing no symptoms (Top) and mild virus-like symptoms 8 weeks from planting.



**Figure 6.** Seed production of two melon lines (Top, Honeydew and bottom, Top Mark) from Cornell University evaluated under field conditions at Sids Experimental Station, Beni Suef, Egypt.

**Objective 2: Evaluation of highly ZYMV-tolerant squash lines containing the cp-gene of ZYMV-CT strain (R5) under open field conditions in different locations in Egypt**

In the period from March to July 2000, 58 R5-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 Sids Experimental Station, Beni Suef, Egypt. Results showed that 11 out of the 58 plants were susceptible for ZYMV infection 12 weeks post planting. In addition, 156 plants of the same line were evaluated for virus resistance evaluation at EL-Nobarria Experimental Station (Figure 7), Egypt. The majority (= 95%) of the tested plants showed no symptoms. This could be due to the epidemiology of the virus in different locations. During the period from September to December 2000, the experiment was carried out under open field conditions in three different locations (El-qanater and Sids Experimental stations as well as AGERI). Similar results were obtained, as the number of resistant plants was higher in Sids followed by El-qanater and AGERI areas. The percentage of

virus-infected plants after 10 weeks from planting was 92, 85 and 78%, respectively. Some plants showed no symptoms after 16 weeks from planting. It is worth mentioning that the transgenic lines produced marketable fruits (Figure 8) and a good yield compared to the non-transgenic plants infected with ZYMV.



**Figure 7.** Evaluation of R5-transgenic squash plants of the highly ZYMV-tolerant line under field conditions at El-Nobaria Experimental Station, Egypt.



**Figure 8.** Marketable transgenic squash fruits of R5-transgenic squash plants of the highly ZYMV-tolerant line produced under field conditions at Sids Experimental Station, Beni, Suef.

**Objective 3: Evaluation of highly ZYMV-tolerant melon lines containing the cp-gene of ZYMV-CT strain (R3) under greenhouse conditions**

36 plants belonging to two R3-transgenic lines of melon (cv. Shahd El-Dokki) named M2000 and M1 were evaluated for ZYMV-resistance in three replicates (6 plants/replicate) under greenhouse conditions at AGERI. Non-transgenic melon plants were also planted and inoculated twice with ZYMV-E strain with seven days interval between each inoculation to serve as a source of ZYMV infection. Another group was also used without virus inoculation to serve as control. Results showed that 2 and 3 plants out of the 18 plants of line M2000 and M1, respectively, showed mild symptoms 12 weeks post planting. Plants were self pollinated for seed production. The results are shown in Figure 9.



**Figure 9.** Two different R3-transgenic lines of melon cv. Shahd El-Dokki evaluated for their resistance to ZYMV-E strain under greenhouse conditions at AGERI. Left, line M1 and right, line M2000.

**Objective 4: Isolation, identification and purification of watermelon mosaic potyvirus (WMV)**

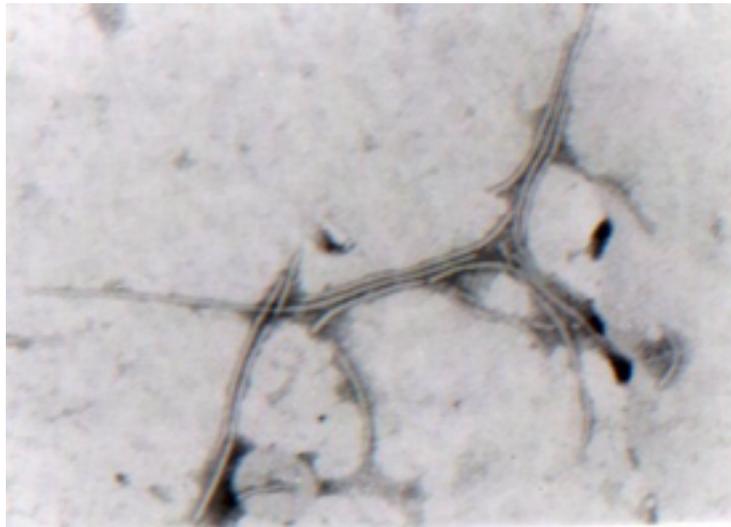
Leaves of watermelon plants cv. Giza 1 showing the characteristic symptoms (Figure 10) of watermelon mosaic potyvirus (WMV) were collected from the open field at Sids Experimental Station. The virus was identified based on symptoms, mode of transmission, differential hosts (Figure 11), stability, inclusion bodies and morphology of virus particles in partial purified virus preparations (Figure 12).



**Figure 10.** WMV-characteristic symptoms on cv. Watermelon plants cultivated under field conditions at Sids Experimental Station.



**Figure 11.** Squash leaves showing development of WMV-characteristic symptoms after 10-15 days from inoculation with sap extracted from WMV-infected watermelon plant. H: healthy leaf.



**Figure 12.** Electron micrograph of filamentous WMV particles stained with 2% uranyl acetate and partially purified from WMV-infected squash leaves after 21 days from inoculation with the infectious sap.

**Objective 5: Isolation, cloning and sequencing the coat protein gene of WMV for construct preparation**

Reverse transcriptase-polymerase chain reaction (RT-PCR) was conducted using the RNA extract prepared from the purified virus and two specific primers for the coat protein (*cp*) gene of WMV. The *cp*-gene will be cloned, sequenced and a construct containing the *cp*-gene and the *bar* gene as a selectable marker will be constructed. This cassette will be used for *Agrobacterium*-mediated transformation of watermelon with the objective of producing transgenic watermelon plants resistant to WMV. The cross protection between the ZYMV and WMV will be also under investigation.

**Objective 6: Establishment of regeneration system in watermelon**

To establish a regeneration system via organogenesis in watermelon cultivars (Giza 1 and Giza 21), six different culture media were tested with the cotyledon segments as explants. Figures 13 and 14 show the different steps in regeneration and acclimatization of watermelon.



**Figure 13.** Organogenesis regeneration of two watermelon cultivars (Giza 1 and Giza 21).



**Figure 14.** Acclimatization of regenerated watermelon plants. Plantlets in Hogland solution (Left) and the elongated ones with stronger roots in pots (Right).

### **Objective 7: Establishment of transformation system in watermelon**

An experiment using different concentrations of kanamycin (25, 50, 75, 100, 125 and 150 mg/l) in the regeneration medium was carried out. Results showed that 100 mg/l kanamycin was effective for selection of putatively transgenic material. The pBI121 plasmid containing GUS and NPT-II reporter and selectable genes, respectively, was used to establish the transformation system *via Agrobacterium*-mediated gene transfer system. Successful transformation events were then confirmed by PCR and/or Southern blot hybridization techniques.

### **Discussion/Implications**

The field trails conducted at four locations, Giza (AGERI), Beni Suef (Sids), Kalubia (Qanater), and El-Behara (El-Nobaria) have verified that the transgenic squash line Eskandarani is highly tolerant to ZYMV infection while maintaining high yield and superior marketable fruit quality. Therefore, the technology transfer office should look into the requirement for its commercialization. Similarly transgenic cantaloupe cv. Shahd El-Dokki should also be considered.

## Highlights of significant achievements

- ❑ Establishment of regeneration and transformation systems in the Giza 1 and Giza 21 cultivars of watermelon.
- ❑ Isolation, identification and purification of WMV followed by isolation of the cp-gene of this virus.
- ❑ Evaluation of virus resistance in the multiple virus resistant squash, melon and cucumber lines obtained from Cornell University under field conditions at Sids Experimental Station.
- ❑ Evaluation of highly ZYMV-tolerant squash lines containing the cp-gene of ZYMV-CT strain (R5) under open field conditions in different locations in Egypt.
- ❑ Evaluation of highly ZYMV-tolerant melon lines containing the cp-gene of ZYMV-CT strain (R3) under greenhouse conditions.

## Publications during the reporting period

S.M.Khalil, M.A.Badawi, Kh.A.Abdel-Ati and M.A.Madkour (2000). Plant regeneration via somatic embryogenesis from cotyledons in cucumber (*Cucumis sativus* L. cv. Beit Alpha). Arab Journal of Biotechnology 3(1):87-96.

## Travel of project personnel and visits by Project partners

Dr. Molly Kyle Jahn, and her group, from Cornell University as well as Mohamed Saleh Tawfic from Dr. Rebecca Grumet's group, Michigan State University visited our laboratory at AGERI, Egypt in May 2000. Dr. M. Kyle Jahn's group visited the Sids Experimental Station, Beni Suef, Egypt to see the Cornell-bred resistant materials (squash, melon and cucumber) grown under Egyptian open field conditions.

## References Cited

- Clough, G.H. and P.B. Hamm (1995). Coat protein transgenic resistance to watermelon mosaic and zucchini yellows mosaic virus in squash and cantaloupe. Plant Dis. 79:1107-1109.
- Fitch, J.H. and R. Beachy (1993). Genetically engineered protection against viruses in transgenic plants. Ann. Rev. Microbiol. 47: 739- 763.
- Gonsalves D. and J.L. Silghtom (1993). Coat protein-mediated protection : analysis transgenic plants for resistance in a variety of crops. Sem Virol. 4: 397-405.
- Khalil, S.M., A.S. Sadik, H. El-Doweny and M.A.Madkour (1999). Production of transgenic squash plants resistant to zucchini yellow mosaic potyvirus. Arab Journal of Biotechnology 2(1): 27-44.
- Lomonoscoff, G.P. (1995). Pathogen-derived resistance to plant viruses. Ann. Rev. Phytopathol. 33: 323-343.

Providenti, G., H.M. Manger and A.O. Paulus (1984). Epidemics of zucchini yellow mosaic virus and other cucurbit viruses in Egypt in the spring 1983. *Cucurbit Genet. Crop* 7:78-79.

Sherf, A.F. and A.A. MacNab (1986). Cucurbits. In: *Vegetable Diseases and Their control* (Johy Wiley & Sons, 2nd ed.): 353-379 New York.

Webb, S.E. and S.B. Linda (1993). Effect of oil and insecticide on epidemics of potyvirus in watermelon in Florida. *Plant Dis.* 77:864-869.

# Potato Transformation for Development of Potato Tuber Moth Resistance

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## Research Team

David Douches, Walter Pett and Edward Grafius, Michigan State University

## Project Partners

Taymour El-Nasr, AGERI, Egypt

## 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:

- › Continue genetic engineering development with emphasis on genes available for commercial development.
- › Continue transformations with new gene/vectors for varieties important to Egypt.
- › Examine the individual foliar expression levels of new transformations in lab and field tests.
- › Evaluate the effectiveness of tubers stored in nawallas in controlling potato tuber moth.
- › Evaluate the effectiveness of individual and combined resistance factors under laboratory experiments.
- › Develop linkages with US companies and Egyptian seed companies to promote the commercialization of desired potato lines.
- › Evaluate different management strategies for maintenance of resistant potato varieties and their integration into IPM systems.

## Importance of the problem

Potato is one of the most important vegetable crops in Egypt with total production up to 2 million tons annually. It is also the leading export vegetable in Egypt, with approximately 225,000 tons exported to the United Kingdom and western European countries. Potato is cultivated in the Nile delta region on about 34,000 hectares. Most of the exported potato production is centered in Behira, Menofya and Garbiya, where yields in these three governates range between 42.5 and 58.5 tons/hectare (Hasan 1991).

The primary insect pest in Egyptian potato production, like many other countries in the Middle East, is the potato tuber moth, *Pthorimaea operculella* (Zeller). In the field, the moths lay their eggs on the potato foliage and the hatched larvae mine the foliage and the stems. This feeding damage leads to irregular transparent tunnels in the leaves and weakening of the stem. The larvae attack the tubers through infected stems or directly from eggs, which are oviposited on exposed tubers or where soil cracks allow moths to reach the tubers. Larvae mine the tuber in the field and in storage reducing potato quality and increasing the potential for pathogen infection. In Sudan, about 30 – 40 % of the potatoes are stored in underground pits and can be completely destroyed by tuber moth within two months (Ali 1993).

## 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 CryV Bt toxin gene has been codon modified to increase its expression level in the plant and transferred into potato. Douches et al. (1998) used different CryV constructs for engineering the potato to express high levels 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 CryV-Bt 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 in the field and in storage. The objectives of this study are to evaluate field-grown CryV-Bt transgenic potato tubers for their resistance to potato tuber moth, and to evaluate 11-12 month old stored transgenic tubers to determine the mortality efficiency of the expressed CryV-Bt toxin.

These transgenic potatoes will reduce the use of insecticides in Egypt 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 CryIa(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.

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'Lemhi Russet', 'Atlantic', L235 - (glandular trichome line), and USDA838-1 (foliar leptine line) (Westedt et al. 1998). The CryV-Bt constructs that differ in the promoter (CaMV 35S, Gelvin super promoter and patatin promoter) were transformed into cv. 'Spunta' (Li et al. 1998a). The Cry5-PVYcp gene construct was also transformed into 'Spunta' (Li et al. 1998b). Spunta is the most important cultivar grown in Egypt, while Atlantic is a desired chip-processing cultivar. Other constructs that have the GUS gene removed are ready to use in transformation. Samples of the CryI and Cry5-t transgenic lines were 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 CryIa-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 Cry5-Bt-Spunta and Cry5-Bt/PVY-Spunta with high levels of potato tuber moth mortality (Li et al 1998a). Other Cry5-Bt-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 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 perform 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, the 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).

A 1999 field trial was conducted in Egypt at AGERI and CIP-Egypt. The results show excellent control of PTM from several lines including SPG2 and SPG3 that had virtually no potato tuber

moth infestation compared to the nontransformed control lines in our CIP field trial. Storage experiments were conducted with the harvested potatoes and again the SPG2 and SPG3 lines had minimal infestation for nearly 3 months in storage.

## Specific Objectives

- › Continue genetic engineering development with emphasis on genes available for commercial development.
- › Continue transformations with new gene/vectors for varieties important to Egypt.
- › Examine the individual foliar expression levels of new transformations in lab and field tests.
- › Evaluate the effectiveness of individual and combined resistance factors under laboratory experiments.
- › Evaluate the effectiveness of tubers stored in nawallas in controlling potato tuber moth.
- › Develop linkages with US companies and Egyptian seed companies to promote the commercialization of desired potato lines.
- › Evaluate different management strategies for maintenance of resistant potato varieties and their integration into IPM systems.

## Research progress

In 2000 field trials were conducted in Egypt at AGERI and at CIP-Egypt. The results show excellent control of PTM from several lines with SPG2 and SPG3 having minimal numbers of mines in the foliage and tubers at harvest. Storage experiments were conducted with the harvested potatoes and preliminary results show that the transgenic 'Spunta' lines had very few infested tubers compared to the nontransformed controls.

Seed production increases for year 2001 field trials in Egypt were made at the MSU Potato Research Center, Montcalm Co., MI of the following 'Spunta' lines; SPG2, SPG3, and SP6a-3 [PVYcp/Cry5]. Our plans are to test these lines in Egypt for seed increase and a large-scale field test at the CIP Station in year 2001.

Agronomic trials were conducted in Michigan testing the Cry5-Bt Atlantic lines. All lines were comparable to the nontransformed control.

New constructs using four different promoters for BtCry5 expression were developed. The different promoters include CAMV35s, Gelvin super promoter (GSP), Potatin, and Ubiquitin3. The Ubiquitin3 promoter was developed by USDA 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 'Spunta' lines transformed with the different constructs.

New constructs using the same four promoters have been developed for codon-modified Cry1Ac (J. Kemp, NMSU) expression. Potato transformation will begin in the near future.

'Atlantic', 'Lady Rosetta' and MSG274-3 (MSU late blight resistant line) transformations with a Cry5 Gus minus vector have been completed. These lines are important chip varieties in Egypt and are key for Egyptian commercialization.

Transformed tissue culture plantlets of 'Spunta' and 'Atlantic' expressing Cry5 were sent to The International Potato Center (CIP), Lima, Peru for testing their populations of PTM. These tests are almost complete and results will be reported.

## 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. This is an important step to commercialization as it allows the producer the opportunity to observe first hand the benefits of the product. Transforming 'Atlantic' and 'Lady Rosette' is also an important step towards commercialization in Egypt as these varieties are very important in the Egyptian chip industry.

## Highlights

The field tests in Egypt have provided useful information about the effectiveness of our transformed potatoes in controlling potato tuber moth. We have also sent tissue culture plantlets of our most promising lines to ARC in South Africa for laboratory testing and field-testing.

## Publications

Pett, W., D. Douches and E. Grafius. 2000. Insect control: Durability and breakdown of resistance. In: *Proceedings of the International Workshop on Transgenic Potatoes for the Benefit of Resource-Poor Farmers in Developing Countries*. Manchester, United Kingdom. pg. 82-86.

## Travel

- › Dr. W. Pett, Dr. D. Douches and Dr. E. Grafius traveled to AGERI, Egypt May/June, 2000 for the ABSP Symposium and to harvest potato field trials.

- › Dr. W. Pett attended a meeting in Manchester, England (June 5 - June 9, 2000) titled: "*International Workshop on Transgenic potatoes for the benefit of resource-poor farmers in developing countries*", presented 2 papers and co-chaired a working group labeled "*Health and food safety*".
- › Dr. W. Pett traveled to South Africa (ARC-Roodeplaat) in September (Sept. 9 – 14) to meet with ARC personnel and people at SA Government Agencies with the intention of initiating a working group consisting of ABSP (potato group) and ARC.
- › Dr. E. Grafius traveled to Basle, Switzerland.

# Managing Natural and Engineered Resistance in Potato to Potato Tuber Moth

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## Research Team (AGERI)

Prof. Magdy Madkour  
Dr. Taymour Nasr El-Din  
Dr. Emad Anis

## Project Partners (MSU)

Dr. Dave Douches  
Dr. Edward Grafius  
Dr. Walter Pett

## 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. The insect attacks potato plants in two ways: by mining the foliage and also feeding on tubers, making it an important pest both in field and storage.

## Objectives

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:

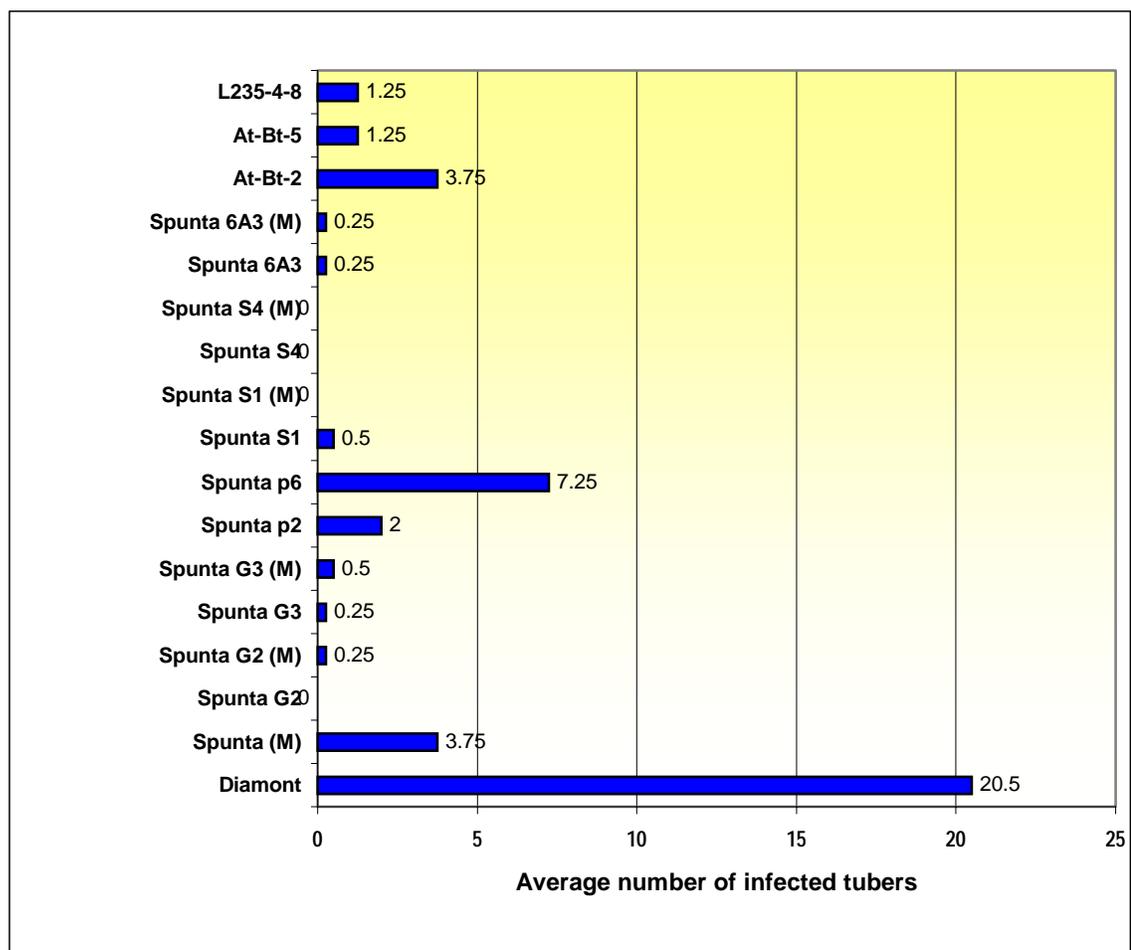
ABSP project provided Bt. transgenic potato lines to test their resistance to PTM in field trials in Egypt after obtaining clearance from USAID, Egyptian National Bio safety Committee (NBC) and CIP- Lima. One trial was carried out at AGERI Experimental plot and the other was conducted at CIP station in the middle of the Delta for evaluation of the level of resistance to potato tuber moth (PTM). Field trials conducted in 2000 involved transgenic lines with different Bt -constructs and various gene promoters.

The lines were derived from cultivars Atlantic, a processing cultivar and Spunta a tuber stock cultivar. Field studies for testing transgenic and non-transgenic lines were conducted in February 2000. Potato tubers were planted in a randomized complete block design with 4 replications. Treatments included transgenic with various modified and non-modified gene constructs. The insect damage on foliage was evaluated 3 times during the growing season. The trials were harvested on June 2000 and tubers were examined for PTM damage

Results of tuber infestation at CIP are presented in Table (1) and Fig.1. Data shows that Spunta lines viz. Sp G2, Sp G3, Sp S1, Sp S1 (M), Sp S4 and Sp S4 (M) were the most promising lines and the Atlantic lines were significantly different compared to control.

**Table (1):** Percentage of infested tubers in field trial harvested in June 2000 at CIP.

Lines	Total number of tubers	Number of Infested tubers	% Infested tubers
Diamont	317	82	25.9
Spunta(M)	97	15	15.47
Spunta G2	151	0	0
Spunta G2(M)	114	1	0.88
Spunta G3	119	0	0
Spunta G3 (M)	118	2	1.69
Spunta p2	159	8	5.03
Spunta p6	188	2	15.42
Spunta S1	101	0	0
SpuntaS1 (M)	105	0	0
Spunta S4	157	0	0
Spunta S4 (M)	50	0	0
Spunta 6A3	195	1	0.51
Spunta 6A3 (M)	108	1	0.93
Atl-Bt-2	263	15	5.7
Atl-Bt-5	116	5	3.0
L235-4-8	309	5	1.62



**Fig (1):** Average number of infested tubers for Spunta, Atlantic, L235-4 and Diamond lines.

Meanwhile, the performance of transgenic lines i.e., agronomic characteristics, were evaluated under field conditions at CIP site. Observations were recorded during the growing season. In general there were no difference between transgenic lines and non-transgenic control lines in terms of Shape-Size-Color of harvested tubers.

The results obtained from field trials at AGERI site was excluded due to low natural insect pressure of PTM at that site. The tubers saved from the previous experiment (1999 season) were planted in a separate plot at AGERI. It was observed that the yield and quality of the tubers produced was very poor because of virus and other disease infections, but they still had resistance to PTM.

The main objective of the trials at CIP site was to select the lines having the highest resistance against PTM among transgenic lines tested. It is well known that the natural pressure of PTM at

CIP site (old land) is much higher versus new cultivated area. Meanwhile, the results indicated the stability of Bt gene expression under high pressure of insect infestation. Therefore, we are going to focus only on three transgenic lines of Spunta (Sp G2, Sp G3 and Sp 6a3) to be tested at CIP station in the next growing season (2001).

The agronomic performance of these transgenic lines will be considered as well and compared to non-transgenic control cultivars under field condition. Previous results revealed that the transgenic lines of Spunta and Atlantic were true to type.



Fig (2): harvest of potato transgenic trials at Kafr El-Zyat (CIP site) in June 2000.

### **Storage Experiment**

Healthy tubers presenting the transgenic and non-transgenic lines were stored in a traditional storage facility (Nawalla) that has no cooling system. Tuber damage was examined during the storage period. Three inspections were carried out at one-month interval. Percentage of rotted and infested tubers was recorded and Table (2) represents the results of inspection. The results revealed that the control lines i.e. Atlantic, Spunta, and Diamant are more susceptible to infestation compared to transgenic lines.

**Table (2):** The percentage of infestation with PTM under Nawalla storage facility.

Potato lines	Number of tubers	1 <sup>st</sup> inspection		2 <sup>nd</sup> inspection			3 <sup>rd</sup> inspection		
		Rotted tubers%	Infested tubers%	Number of tuber	Rotted tubers%	Infested tubers %	No. of tuber %	Rotted tubers %	Infested tubers%
Spunta (A)	78	6.9	16.1	67	2.9	26.8	65	0.0	10.7
Spunta 6A(M)	148	2.7	2.7	140	0.7	9.3	139	0.0	1.4
Spunta -S4	134	2.2	8.2	114	0.0	7.0	114	0.0	2.6
L235-4-8	188	1.6	0.0	185	0.0	3.8	185	0.0	0.5
Spunta- p6	207	4.4	8.2	178	2.8	21.3	173	0.0	28.9
Spunta 6A-3	225	2.2	0.0	220	1.4	4.1	217	0.0	0.9
Spunta G2 (M)	123	0.0	2.4	120	0.8	2.5	119	0.0	0.0
Atl-Bt2	300	0.0	3.3	290	0.3	8.6	289	1.4	3.1
Spunta –Bt2	302	0.66	3.3	290	0.0	7.2	290	1.0	1.0
Spunta 6 A3(M)	163	1.8	0.0	160	1.9	1.9	157	0.6	3.2
Spunta G2	138	3.6	2.2	130	0.8	1.5	129	0.8	3.1
Spunta S1 (M)	180	5.6	0.0	170	0.0	3.5	170	0.6	2.9
Atl-Bt5	165	0.0	3.0	160	0.0	5.6	160	0.0	4.4
Spunta S4 (M)	404	0.99	0.0	400	0.0	1.8	400	0.0	1.5
Spunta G3	205	1.5	0.0	200	1.5	3.5	197	0.5	0.0
Spunta S1	203	1.5	0.0	200	0.0	2.0	200	0.5	0.0
Dimont	110	2.7	4.5	102	11.8	19.6	90	5.6	33.0

### Bio-assay of PTM

This experiment was conducted to measure 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 an insect proof container till emergence of the moths. Numbers of non –transgenic tubers from different lines were saved as control. After 28 days, number of emerging PTM moths were counted (Table 3) and compared to control. Data indicated that even under very high insect infestation (50 larvae/tuber) the gene expression is working perfectly specially in Spunta transgenic lines.

**Table (3):** Inhibition percentage of the adult moth emergence in transgenic and non-transgenic potato tubers.

Lines	Number of adult moth	% Moth inhibition from emergence ( I )
Spunta (Control)	21	0
SP-A6 <sub>3</sub> (M)	8	62
Sp-P <sub>6</sub>	18	14.3
SP-P <sub>2</sub>	16	23.8
SP-S <sub>1</sub> (M)	0	100
Sp-S <sub>1</sub>	1	95.2
SP-G <sub>3</sub>	0	100
SP-G <sub>2</sub> (M)	1	95.2
SP-S <sub>4</sub>	0	100
SP-S <sub>4</sub> (M)	0	100
SP-G <sub>2</sub>	0	100
SP-G <sub>3</sub> (M)	0	100
Atlantic (Control)	24	0
At-Bt <sub>2</sub>	8	66.66
At-Bt <sub>5</sub>	4	83.33

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

## Conclusion

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.

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. To commercialize the transgenic potato tubers in future for local consumption we have a problem with the presence of the antibiotic resistant gene. Therefore, we should shift to using the *Bar* gene instead of kanamycin gene. The well known other problem concerns the exportation of potato from Egypt to Europe and the regulatory issues imposed there on transgenic material.

We are planning to save the highly promising transgenic lines to be used as stock seeds for future commercialization plans. We are also maintaining these lines at the AGERI gene bank as tissue culture germplasm.

The clearance of transgenic potato for food and feed safety and environmental assessment will be required by the Biosafety committee before taking steps towards the registration of Bt. transgenic lines for commercial release. The help of the Office of Technology Transfer and Intellectual Property at AGERI will be considered through all the steps taken for the commercialization of transgenic potatoes.

# Evaluation of Bt-cryV transgenic potatoes on two species of potato tuber moth, *Phthorimaea operculella* (Zeller) and *Symmetrischema tangolias* (Gyen) in Peru

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## Research Team

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## Introduction

The name potato tuber moth is used to refer to several insects that cause great damage to potato crops in tropical and subtropical regions. For example *Phthorimaea operculella* (Zeller) attacks potato foliage, stems and tubers (Figures 1a and b), although field infestations rarely cause serious yield losses. The main damage caused by this insect is to stored tubers: in warm climates losses can reach 100%. *Symmetrischema tangolias* (Gyen) is considered a pest of economic importance in potato stores in Peru and Bolivia (Ewell *et al*, 1994), but is a serious pest throughout the Andean region. Larvae burrow into tubers (Figures 1c and d) making them unfit for human consumption.

Host plant resistance is a desired component of an integrated pest management (IPM) program to control potato tuber moths. *Bacillus thuringiensis* (Bt) toxin genes have been cloned, codon-modified, and inserted in various crop species (Barton and Miller, 1993). Transgenic potato varieties with the insecticidal crystal protein Cry1Ab offer high levels of resistance to *Phthorimaea operculella* (Jansen *et al*, 1995; Cañedo *et al*, 1999) in foliage and tubers (field and stores). A Bt-cryV toxin gene, with activity against Lepidoptera and Coleoptera, was codon-modified to increase its expression level in the plant. Douches *et al* (1998) transformed this gene into potato to achieve control of potato tuber moth, *P. operculella*, obtaining a high expression level of Bt-CryV protein in the leaves and up to 96% mortality of potato tuber moth larvae in foliar assays.

This paper describes a study of larval development of two potato tuber moth species on CryV Bt transgenic potato plants in Peru.

## Material and methods

The transgenic material used in this study was developed at Michigan State University, USA. Four lines of cv. Atlantic and five lines of cv. Spunta were transformed with a codon-modified *cryV-Bt* gene using an *Agrobacterium*-mediated technique.

### Transgenic lines (Table 1):

Atlantic transgenic lines contain the *cryV-Bt*/GUS ( $\beta$ -glucuronidase) gene fusion with the CaMV35S promoter. Two Spunta lines were transformed with the *cryV-Bt* gene controlled by the CaMV35S promoter (pBIML5-vector). Two other Spunta lines were transformed with the *cryV-Bt* gene controlled by the Gelvin Super promoter (pBIML1-vector) (Mohammed *et al*, 2000). One Spunta line was transformed with the *cryV-Bt*/35S/PVY gene fusion controlled by the CaMV35S promoter (pBIML6a-vector) (Li *et al*, 1999).

A detached leaf bioassay was used to test the mortality of potato tuber moths living on *CryV-Bt* transgenic plants. The petiole of the leaf was inserted in a sponge fitted in the top of a glass vial full of water. The leaf and the vial were then placed on a filter paper disk in a petri dish (25 x 150 mm). Ten neonate larvae were introduced into each petri dish. Each petri dish was considered as a replication and five replications per transgenic line were used in a completely randomized design.

Survival of the larvae was recorded. For *Phthorimaea operculella*, evaluations were made at 3 and 8 days after infestation. For *Symmetrischema tangolias*, one evaluation was made 5 days after infestation. Larval development on the transgenic lines was assessed by measuring the average length of larvae growing on the transgenic lines, compared with the length of larvae growing on the control plants.

### Statistical analysis

All mortality data were subjected to arcsine transformations before analysis. Data from the two cultivars were analyzed separately. Means were compared using the Waller-Duncan test. The statistical program SAS for Windows was used for statistical analysis.

## Results and Discussion

Mortality of *P. operculella* on Atlantic transgenic lines after 3 days of infestation was low (4 to 26%) and generally similar to that on the non-transgenic control. After 8 days, insect mortality on two lines was significantly ( $P=0.01$ ) higher than on the control (Figures 2 and 3a). All Spunta transgenic lines showed high levels of resistance to *P. operculella*; insect mortalities ranged from 72 to 85% after 3 days and from 80 to 98% after 8 days (Figures 2 and 3b), and were all significantly ( $P=0.001$ ) different from mortality on the non-transgenic control. The reduction in larval length ranged from 61 to 66.3% on Atlantic and from 46.6 to 89.5% on Spunta lines (Table 2).

Larval mortality of *S. tangolias* showed big differences across Atlantic transgenic lines. Mortalities on two lines (Bt-2 and Bt-5) were significantly ( $P=0.001$ ) higher than that on the control (Figures 4 and 5a).

Mortalities of *S. tangolias* larvae were significantly ( $P=0.001$ ) higher than that on the nontransgenic control (Figures 4 and 5b). Larval development was severely affected on both transformed cultivars. The reduction in larval length ranged from 52.3 to 85.7% on Atlantic and from 77.4 to 87.9% on Spunta lines (Table 3).

Of the potato tuber moths grown on Atlantic transgenic lines, *S. tangolias* larvae were generally more affected than were *P. operculella* larvae.

Li *et al* (1999) noted that the Spunta G-3 transgenic line contains significantly more CryV-Bt protein than does Spunta G-2, this may explain why the larval mortality on G-3 is higher than on G-2 for both species.

## Conclusions

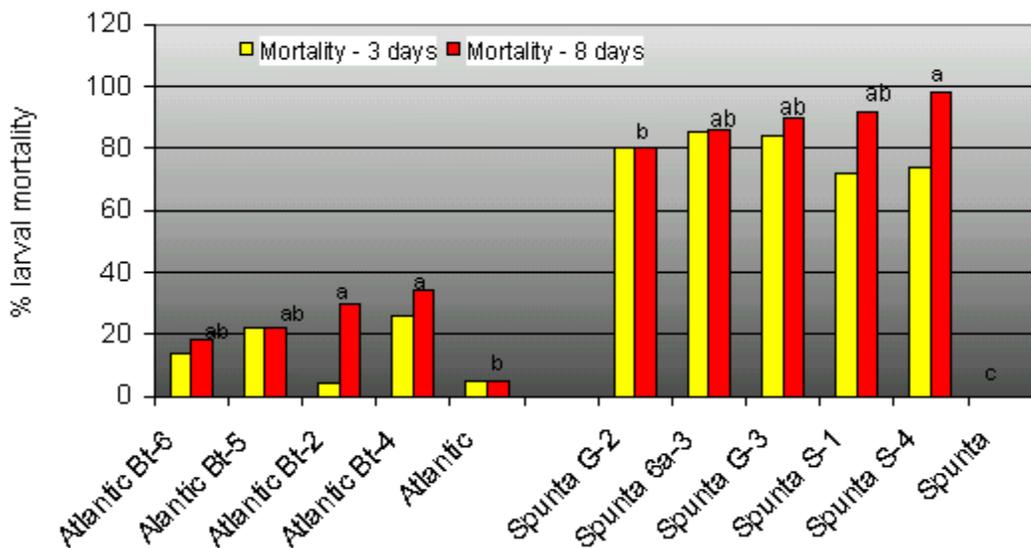
Mortality of both *P. operculella* and *S. tangolias* was found to be higher on leaves of transgenic Spunta lines.

This *cryV-Bt* gene therefore appears to offer another source of resistance genes, which can be pyramided for effectiveness toward the development of durable resistance to potato tuber moths and other insect pests. Future work will test mortality of these insects on transgenic tubers.

**Figure 1.** a) Foliage damage by *Phthorimaea operculella*, and adult insect. b) Tuber damaged by *P. operculella*. c) Tuber damaged by *Symmetrischema tangolias*. d) Adult of *S. tangolias*.

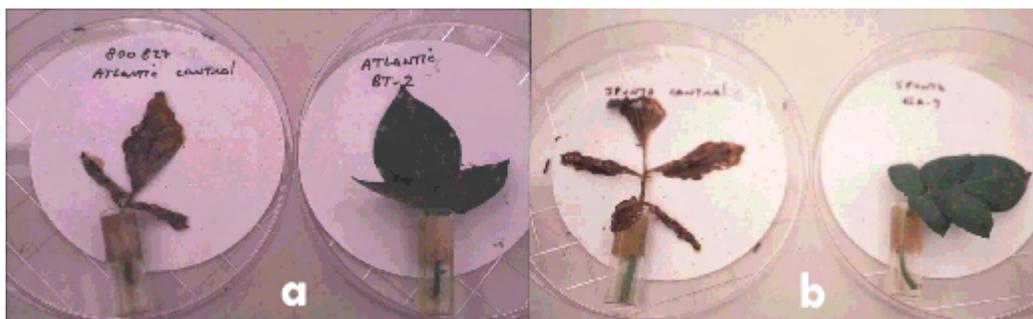


**Figure 2:** Mortality of *Phthorimaea operculella* on Cry V-Bt transgenic Atlantic and Spunta lines, 3 and 8 days after infestation

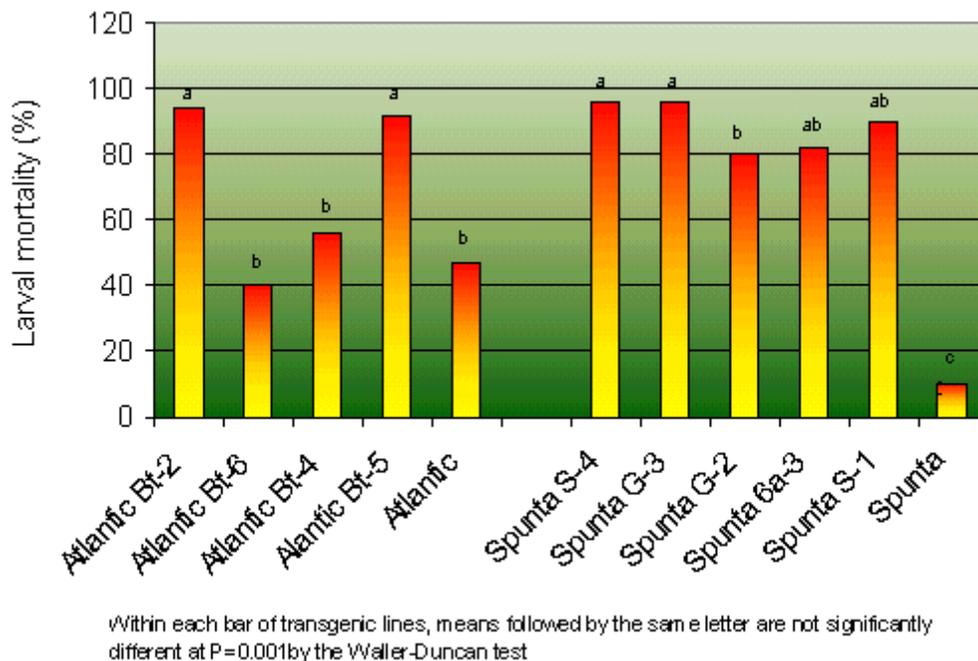


For mortality at 8 days, within each group of transgenic lines columns with the same letter are not significantly different at  $P = 0.01$  (by the Waller-Duncan test)

**Figure 3:** a) Atlantic: Untransformed control (left) and transgenic line (right) damaged by *Phthorimaea operculella*. b) Spunta: Untransformed control (left) and transgenic line (right) damaged by *P. operculella*



**Figure 4:** Mortality of *S. tangolias* on Cry V-Bt transgenic Atlantic and Spunta lines, 5 days after infestation.



**Figure 5:** a) Untransformed control (left) and transgenic Atlantic line (right) damaged by *Symmetrischema tangolias* b) Untransformed control (left) and transgenic Spunta line (right) damaged by *S. tangolias*



**Table 1.** Transgenic potato lines evaluated for control of potato tuber moth bioassays and their *cryV*-Bt gene constructs.

Line	Construct	Vector
All Atlantic- <i>Bt</i> lines	35S/ <i>cryV</i> -Bt/GUS	pB1cry5
Spunta S-1	GSP/ <i>cryV</i> -Bt	pBIML1
Spunta S-4	GSP/ <i>cryV</i> -Bt	pBIML1
Spunta G-2	35S/ <i>cryV</i> -Bt	pBIML5
Spunta G-3	35S/ <i>cryV</i> -Bt	pBIML5
Spunta 6a-3	35S/ <i>cryV</i> -Bt/35S/PVY	pBIML6a

**Table 2.** Reduction of *P. operculella* larva length in Atlantic and Spunta *CryV*-Bt transgenic lines.

Transgenic lines	% reduction*	SD
Atlantic Bt-2	61.0 a	1.03
Atlantic Bt-6	65.9 a	0.90
Atlantic Bt-4	66.3 a	0.80
Atlantic Bt-5	65.7 a	0.81
Atlantic (control)	0 b	0.81
Spunta S-4	89.5 a	0.72
Spunta G-3	49.8 b	2.14
Spunta G-2	46.9 b	1.48
Spunta 6a-3	63.3 b	1.34
Spunta S-1	46.6 b	1.98
Spunta (control)	0 c	1.08

\* Within each group of transgenic lines, means followed by the same letter are not significantly different at P= 0.001 (by Waller-Duncan test)

**Table 3.** Reduction of *S. tangolias* larva length in Atlantic and Spunta CryV-Bt transgenic lines.

Transgenic lines	% reduction*	SD
Atlantic Bt-2	85.7 a	0.0
Atlantic Bt-6	76.0 a	0.19
Atlantic Bt-4	71.4 a	0.0
Atlantic Bt-5	52.3 b	0.58
Atlantic (control)	0 c	0.85
Spunta S-4	87.9 a	0.02
Spunta G-3	83.7 a	0.13
Spunta G-2	77.8 a	0.12
Spunta 6a-3	77.4 a	0.11
Spunta (control)	0 b	2.07

\* Within each group of transgenic lines, means followed by the same letter are not significantly different at P= 0.001 (by Waller-Duncan test)

## References

- Barton K and Miller M. 1993. Production of *Bacillus thuringiensis* insecticidal proteins, pp 297–315 In Kung S and Wu R [eds]: Transgenic plants, vol.1 Engineering and utilization. Academic Press, San Diego, CA, USA.
- Cañedo V, Benavides J, Golmirzaie A, Cisneros F, Ghislain M and Lagnaoui A. 1999. Assessing Bt-transformed potatoes for potato tuber moth, *Phthorimaea operculella* (Zeller), management. pp 161–170 in: Impact on a changing world. Program Report 1997–98. International Potato Center (CIP) Lima, Peru.
- Douches D, Wested A, Zarka K, Schroeter B and Grafius E. 1998. Potato transformation to combine natural and engineered resistance for controlling tuber moth. HortScience 33(6):1053–1056.
- Ewell P, Fano H, Raman KV, Alcázar J, Palacios M and Carhuamaca J. 1994. Farmer management of potato insect pests in Peru: Report of an interdisciplinary research project in selected regions of the highlands and coast. International Potato Center (CIP), Lima, Peru. 77 p.

- Jansens S, Cornelissen M, De Clerco R, Reynaerts A and Peferoen M. 1995. *Phthorimaea operculella* (Lepidoptera: Gelechiidae) resistance in potato by expression of the *Bacillus thuringiensis* Cry1A(b) insecticidal crystal protein. *Journal of Economic Entomology*. 88(5):1469–1476.
- Li W, Zarka KA, Douches D, Coombs J, Pett W and Grafius, J. 1999. Coexpression of potato PVY coat protein and *cry-Bt* genes in potato. *Journal of the American Society of Horticultural Science* 124(3):218–223.
- Mohammed A, Douches D, Pett W, Grafius E, Coombs J, Li L and Madkour M. 2000. Evaluation of potato tuber moth (Lepidoptera:Gelechiidae) resistance in tubers of *Bt-cry5* transgenic potato lines. *Journal of Economic Entomology* 93(2): 472–476.

# Tomato transformation for Development of Geminivirus Resistance

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## Problem Definition

In Egypt, tomatoes are cultivated on 170,000 hectares, one-third of the cultivated area devoted to vegetable crops. Tomatoes are considered an export crop, so it contributes significantly to the Egyptian economy. In 1999, the total world production of tomatoes was estimated to be 95 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).

Whitefly-transmitted geminiviruses (WTGs) are a major threat to the productivity and quality of tomato grown in subtropical and tropical regions of the world. Recently, the outbreak of whitefly populations has been recorded as a result of the development of resistance to insecticides that are used to control geminivirus vectors. Viruliferous whiteflies, these physically tiny but agriculturally huge pests have inflicted massive losses on different crops. Yield and quality are negatively affected as a result of direct feeding damage and numerous plant diseases transmitted by whitefly. Although whiteflies have been recognized since 1930s, they have become very important pests during the last ten years. In Egypt, the most severe damage is associated with infections occurring in April - November planting which overlap with production of cotton and other vegetables. At least twenty-one aleyrodid pests have been documented in the Egyptian cropping system; three of them (*Bemisia tabaci*, *B. argentifolii* & *Trialeurodes ricini*) are able to transmit geminiviruses. TYLCV is economically significant whitefly-transmitted geminivirus. It causes about 65% yield losses in tomato (Table 1). Geminiviruses were discovered in 1977. Today there are at least 77 geminiviruses described worldwide; this number rings an alarm. These viruses cause devastating plant diseases to many crops in Egypt (tomato, clover, cotton, cucumbers, melon, faba bean, altheae).

**Table 1:** Major food and fiber crops grown in the open field and under protected cultivation and affected by the whiteflies-viruses complex in Egypt.

Crops	Open Field			Protected Cultivation		
	Cultivate Area (ha)	Affected Area (ha)	Losses %	Cultivated Area (ha)	Affected Area (ha)	Losses %
Beans	23.000	4.000	3%	68	42	3%
Citrus	160.000	200	5-10%	---	---	---
Cotton	820.000	125.000	4%	---	---	---
Cucumber	226.000	126.000	5%	888	205	12-30%
Eggplant	20.000	8.000	5%	---	---	---
Melon	44.000	6.000	2%	70	30	5-15%
Potato	88.000	12.000	1%	---	---	---
Squash	18.000	6.000	5%	---	---	---
Sweet potato	40.000	6.000	10%	---	---	---
Tomato	176.000	120.000	65%	60.000	1.500	5%
Watermelon	181.000	80.000	12%	---	---	---

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. Cultural control is difficult due to the wide variety and year-round availability of cultivated crop and weed hosts that serve as reservoirs to the whiteflies. It is well established that, whiteflies quickly develop insecticidal resistant strains partly, because they can produce a new generation about every 3 weeks. Moreover, whitefly-feeding behavior has induced many problems associated with insecticide application. In order to avoid further environmental degradation resulting from extensive use of chemical insecticides, alternative strategies utilizing biotechnology advances will be evaluated. Molecular genetics and plant transformation techniques provided new tools to introduce foreign genes into plant tissues without compromising other economic characters of the existing cultivar. Viral derived genes were used to engineer tomatoes for viral resistance.

## General Objectives

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

## Technical Approaches

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 in Egypt are:

1. Expression of antisense RNA against complementary sense gene.
2. Over expression of coat protein in transgenic plants.
3. Over expression of movement protein in transgenic plants.
4. Expression of a virus induced cytotoxin gene in transgenic plants.

## Achievements

### **Development of a recombinant DNA construct for resistance against tomato yellow leaf curl virus based on sense or antisense expression of C<sub>1</sub> 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 replication within the conserved nonanucleotide TAATATTAC and ATPase activity indispensable for viral DNA replication. Two different constructs were designed and used for transforming tomato plants; *rep* gene in the antisense orientation under the control of e35S promoter, with or without TEV-leader and dominant negative mutation by introducing mutation in domains responsible for the ATPase activity and the DNA cleavage sites. Expressing the antisense-*rep* gene in tomato gave a degree of resistance and transgenic plants were evaluated until T<sub>3</sub> generation. However, the level of resistance obtained from these plants was not stable and was affected by the environmental conditions. While dominant mutation gave a phenotypic abnormality due to the high expression of the movement protein (C4) gene in plants as well. Therefore, it is necessary to eliminate the *c4* start codon before expressing this construct.

### **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 its over expression causes a degradation of cytoplasmic RNA and death of the cell. The expression of RNase-barnase is driven by viral coat protein promoter and cloned in the plant binary vector pBin19 as shown in figure (1). 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.

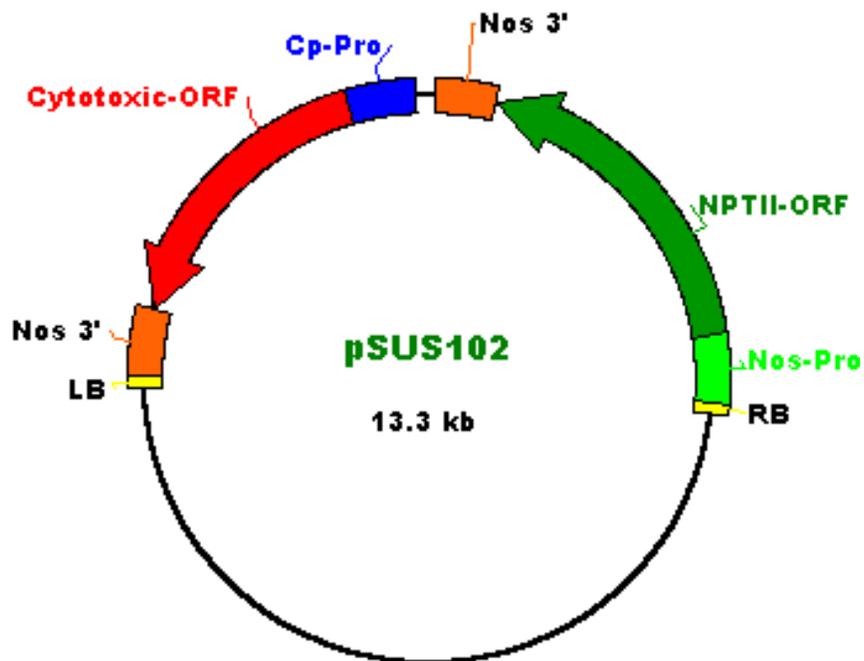
Seeds from T<sub>1</sub> were cultivated in greenhouse biocontainment to produce T<sub>2</sub> plants. Plants were challenged with viruliferous whiteflies with source of infection. Out of 83 transgenic plants, 21 showed severe symptoms while the others were healthy (Figures 2 & 3). The ratio is about 3:1 as expected in the T<sub>2</sub>. DNA was extracted from T<sub>2</sub> transgenic tomato plants and evaluated using PCR/DNA probe (Figure 4). Seeds were collected from T<sub>2</sub> 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 at the end of October 2000. The different lines and the control randomly distributed in the greenhouse. Plants were subjected to natural infestation with the viruliferous whiteflies. Four weeks later, geminiviral symptoms were weekly recorded (Figures 5 & 6). After three months from transplantation, 57 plants out of 180 (31.6%) showed typical TYLCV symptoms.

**Development of a recombinant DNA construct for resistance against TYLCV based on over expression of viral coat protein gene:**

The geminivirus coat protein has a nuclear target sequence that directs the protein into the nucleus. Construct with coat protein gene in the sense orientation was used to transform tomato plants. In the transgenic plants, over expression of the Cp showed no viral resistance. Therefore, this strategy is not recommended to obtain resistance against geminiviruses.

**Development of a recombinant DNA construct for resistance against TYLCV based on over expression of viral C4 (movement protein) gene:**

Movement protein (Mp) of TYLCV through vascular system of tomato plants depends on the expression of the C4 protein. C4-GFP fusion was fused in the viral infectious clone and the plants showed that the fused proteins were expressed in the membrane of vascular cells. Plasmid containing the *c4* gene in the sense orientation was constructed and used to transform tomato plants. Over expression of the C4 protein in transgenic tomato carrying the *c4* gene, causes TYLCV-like symptoms. Therefore, the Mp needs to be modified to block the virus expression and symptoms appearance in transformed plants.



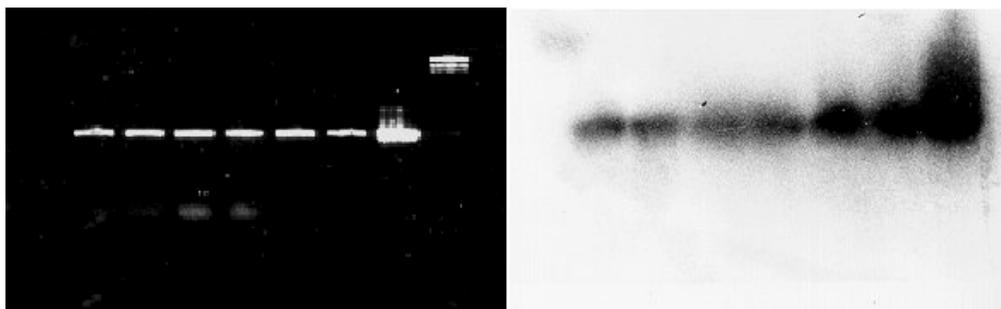
**Figure (1):** Genetic map of the constructed expression cassette carrying the cytotoxic RNase-barnase gene under the control TYLCV coat protein-promoter cloned into the binary vector pBin19.



**Figure 2:** T<sub>2</sub> plants cultivated in the greenhouse biocontainment challenged with viruliferous whiteflies.



**Figure 3:** T<sub>2</sub> transgenic plants show no symptoms after virus infection.



**Figure 4:** PCR/DNA probe evaluation of T<sub>2</sub> plants to detect the introduced construct.

Expression of the C4 protein in transgenic tomato carrying the c4 gene causes TYLCV-like symptoms. C4-GFP fusion was fused in the viral infectious clone and the plants showed that the fused proteins were expressed in the membrane of vascular cells.



**Figure 5:** T<sub>3</sub> plants cultivated in commercial greenhouse challenged with viruliferous whiteflies showing healthy and infected plants.

# Developing Drought and Salinity Tolerant Wheat and Tomato for Egyptian Agriculture

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## Principal Investigator

Prof. D. P. S. Verma, Ohio state University, USA

## Project partners

Dr. Magdy Madkour, AGERI, Egypt

## Project Goal

The overall project goal is to enhance osmotic stress tolerance in wheat and tomato crops by overexpressing proline biosynthesis genes and isolate other genes that can improve osmotolerance in crop plants.

## Importance of the problem and rationale of the approach

Water stress (hyperosmotic) caused by drought and salinity is the most important abiotic factor limiting plant growth and crop productivity worldwide. The 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 the irrigation of an additional two million feddans of cultivated land along the north coast of Egypt. In addition, agricultural development in North Sinaie as well as the Century Project of the New Valley in the western desert "Tushky" will depend mainly on irrigation with mixed fresh and drainage water, which raises the need for developing crop cultivars with increased salt and drought tolerance. The gap between future supply and demand in wheat and tomato makes it imperative to increase cultivation in the areas where sub optimal conditions, such as water deficit, salinity, and high temperature, prevail most of the year.

## 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. We have isolated all of the genes involved in proline biosynthesis and demonstrated that P5CS is a rate-limiting enzyme in proline synthesis and that the over expression of this enzyme produces more proline if sufficient nitrogen is available. We have identified a bifunctional enzyme P5CS able to make P5C from glutamate and shown that the activity of this enzyme limits proline synthesis. Furthermore, we have removed feedback control of this enzyme by proline. This mutagenized enzyme is active up to almost 1M concentration of proline. Recently published data suggest that over expression of P5CS can produce more proline in plants (Hong et al. 2000).

In collaboration with AGERI we are proceeding to genetically engineer wheat and tomato plants with the ability to accumulate high levels of proline, which is expected to confer osmotolerance. This approach is expected to have a major impact on crop productivity in Egypt. After novel genes are identified under this cooperative project, they will be transferred to AGERI for introduction into Egyptian wheat and tomato commercial cultivars. The methodology we will use for wheat and tomato can be considered as a model for other monocot and dicot crops. This technology will be transferred via the OSU technology transfer office to AGERI, which would further develop and commercialize it for Egyptian agriculture. The modified P5CS gene shown to confer drought tolerance (Hong et al., 2000) is being transferred to AGERI for introducing it in the commercial varieties of wheat and tomato.

## Research Progress

Mr. Magdy Mahfouz from AGERI has been working in Dr. Verma's laboratory at the Ohio State University and has been engaged in the mutagenized P5CS gene construct for wheat and tomato transformation. In addition, he has embarked on the following project to isolate novel genes that can confer water stress tolerance by controlling signal transduction pathway.

It has been demonstrated that citrus accumulate significant levels of osmolytes including mainly proline and proline betaine. As a part of this project we used a heterologous functional complementation approach to fish out new genes that might be involved in conferring stress tolerance in crop plants. We used an *E. coli* strain that is pro- and bet- due to the (gpt) lac deletion, which spans both of these loci to complement it with the Citrus cDNA expression library. Citrus cDNA expression library was constructed in lambda zap expression vector. The cDNA library was transformed into *E. coli* lacking bet operon strain using mass in vivo excision protocol. The threshold level of salt tolerance was determined for this particular strain. We screened the library using this threshold level (0.45 M LiCl). We screened more than 500,000 clones. We fished out several clones that exhibit significant salt tolerance in *E. coli*. This led us to the identification of a novel family of universal stress proteins that play a role in stress signal transduction.

## Previous work

### Sequence analysis:

We employed secondary structure homology tool to prove that all genes we have isolated encode proteins that have the same secondary structure with USP. Using conserved domain search tool, we identified a family of USP that contains 19 members.

We used various bioinformatics computer programs to identify phosphorylation sites and additional signaling domains, PDZ domain. PDZ domain is the homodimerization domain and it seems that it is essential for the protein function. Several of these proteins were found to have

PDZ domains suggesting that either they dimerize or interact with other PDZ domain containing proteins.

**Kinase assay:**

Using in vitro phosphorylation assay with labelled ATP we showed that USP is family of a phosphoproteins that may be autophosphorylated or transphosphorylated.

We were also successful in identification of several Arabidopsis universal stress proteins, Usp, cDNAs. Using a conserved domain search program, a family of 19 member of universal stress proteins were identified in Arabidopsis. Usp family is conserved in all archea, most bacteria, plants and fungi. It appears that the UspA family of proteins and domains are nucleotide binding signal transducers that play a central regulatory role, yet to be identified. These proteins confer global stress tolerance by controlling various signal transduction cascades.

**Current work:**

Identification of proteins that interact with USPs (interacting partners) is of crucial importance for this project to nail down the exact molecular function of USPs in signal transduction and stress regulation. Currently we are pursuing a yeast two-hybrid genetic screen to identify protein partners that may be involved in known signaling pathways. We believe that USP family of proteins is a central component of the stress signaling pathway(s), including the control of cation transporter and hence ion homeostasis.

To further analyze the function of USP family member(s), we are pursuing the generation of over and under-expression of Arabidopsis transgenic lines. Since we have a family of these proteins in Arabidopsis, the chances of redundancy are very high. So, in parallel we are conducting experiments on RNA interference, RNAi , to generate the underexpression version of transgenic lines.

**Implications of Results**

Preliminary data suggest that we are able to fishout new genes from Citrus and Arabidopsis that would be valuable for osmotolerance work and these genes once characterized may be considered for patent protection.

**Highlights and Impact**

The identification of novel genes for osmotolerance would have significant impact on the problem of drought and salinity resistance in crop plants and would be highly beneficial to the developing countries such as Egypt.

## Travel

Attended ASPP meeting in San Diego in July 2000.

## Work Plan for Jan 2001- December 2001

Future work would involve precise molecular understanding of the USP signaling pathway and the possible ways to engineer plants to better tolerate adverse osmotic conditions. This will involve genetic studies and biochemical characterization of the proteins involved.

This study is expected to yield some new genes that can be protected by filing appropriate patents. Moreover, this group of genes may elucidate the central mechanism of signal transduction that acts as a global regulator of all stress signals. Progress in this area will be of great significance because it may allow us to control, heat, salt and drought tolerance as well as general oxidative stress tolerance generated by various treatments. Patent application(s) will be filed with Ohio State Research Foundation, in case one or more of these genes turned out to be novel and effective in the bioassay.

Mr Magdy Mahfouz is expected to attend two training courses at Cold Spring Harbor, New York to learn more tools of molecular genetics. These courses of two weeks each would allow him to take full advantage of the emerging technologies in this area of research and build further contacts with people in this field.

# Developing Drought And Salinity Tolerant Wheat For Egyptian Agriculture

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## Research Team

### AGERI

Prof. Dr. Magdy Madkour

Dr. Ahmed Bahieldin

Dr. Ashraf Haider

## Project partners

### The Ohio State University

Prof. Desh Pal S. Verma, Ph.D. FRSC

## Overall project goal

The ultimate goal of this project is to enhance osmotic stress tolerance in Egyptian wheat crop through genetic engineering. This will 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 will be made to find gene(s) able to convert proline into proline betaine.

## Importance of the problem

It is quite important to expand the agricultural system to the drier, less arable land of Egypt to meet the food demands of the growing population. 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 environment. Furthermore, it will provide information for selection of stress tolerance among different wild plant sources. Since wheat is one of the most important pulse crops in Egypt, engineering of stress-tolerance genes to provide varieties that could grow under stress conditions is one of the choice agricultural measures.

## Project background

Wheat is one of the most important field crops worldwide, with the largest harvested area and production levels. 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. Rice was the first major cereal crop transformed by the use of direct DNA delivery into regeneration-competent protoplasts (Toriyama *et al.*, 1988). 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 (Redway *et al.*, 1990; Vasil *et al.*, 1992) from which transgenic plants can be recovered efficiently. The efficient production of fertile transgenic maize (Fromm *et al.*, 1990; Gordon-Kamm *et al.*, 1990), oat (Somers *et al.*, 1992), sugarcane (Bower and Birch, 1992) and wheat (Weeks *et al.*, 1993, Bahieldin *et al.*, 2000) plants was accomplished by using embryogenic suspension cells, immature embryos or callus cultures as target tissues and microprojectile bombardment as the mechanism of DNA delivery. This methodology opened the way to overcome many problems of cereal transformation.

Field crops are often prevented from achieving their full genetic potential by biotic or abiotic environmental stresses. Water stress (hyperosmotic) caused by drought and salinity is the most important abiotic factor limiting plant growth and crop productivity worldwide (Boyer, 1982). 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.

Plants have evolved adaptive mechanisms to cope with the effects of water stress through many physiological responses. A primary response used by plants is the synthesis and accumulation of one, or more, low molecular weight compounds (osmolytes) to maintain equal water potential with the extracellular environment. The best-characterized biochemical response of plant to water stress is the accumulation of organic osmolytes such as proline (Hu *et al.* 1992), polyols such as mannitol (Tarczynski *et al.* 1993). Genetic and transformation data accumulated so far indicate that overproduction of some of these osmolytes results in enhanced tolerance to water stress.

## Rationale for approach

Recent advances in wheat transformation technology (Vasil *et al.* 1993; Weeks *et al.* 1993) and molecular and physiological studies of the genes involved in osmotic responses to stress conditions will allow us to introduce genes to improve water-stress tolerance in Egyptian cultivars. Transformation experiments using these genes in dicotyledonous 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 will use for wheat can be considered as a model for other cereal crops.

## 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 5 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 overexpression 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.

Rice *HAL2*-like (*RHL*) cDNA (Peng and Verma, 1995) has been isolated and characterized by Professor Verma's group. 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. Overexpression of this gene in plants results in accumulation of glutathione, which also reduces oxidative as well as salt stress.

Collaboration between AGERI and Ohio State University started by transforming immature embryos of Egyptian and American bread wheats with Verma's group genes for salt and drought tolerance. Using other genes at AGERI, many putative transgenic plants of both Egyptian and American cultivars have been obtained. In addition, we have been successful in increasing regeneration and transformation efficiencies for both by shortening the selection period, bombarding young immature embryos, using low dicamba concentrations (Bahieldin *et al.*, unpublished) and allowing callus to recover for a week after bombardment (Bahieldin *et al.*, 2000). At AGERI, 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 good expression for the first gene under stress condition. The other one is under test now to evaluate the efficiency of using it in conferring salt tolerance to the Egyptian wheat cultivar Giza 164.

## Specific project objectives

- ❑ Enhance collaborative relationships that will improve modern genetic technology in Egyptian agricultural research
- ❑ Improve transformation and regeneration efficiencies for Egyptian wheat cultivars

- ❑ Develop different protocols for selecting Egyptian transgenic wheat plants
- ❑ Introduce *P5CS*, mutagenized *P5CS* (devoid of feedback control) and *HAL2*-like cDNAs under the control of appropriate promoters in wheat
- ❑ Conduct greenhouse and field performance trials of regenerated transgenic plants
- ❑ Incorporate transgenic plants with improved salt and drought tolerance into ongoing Egyptian breeding programs

## Research progress

Regarding AGERI's transformation plans for this ABSP Project, we have been able to do the following:

### A. Gene construction:

Two plasmids bearing *P5CS* and *Hal2*-like genes were constructed. Besides, two other plasmids were used for co-transformation with *Hal2*-like/*bar* genes. The latter gene (*bar*) is for herbicide-resistance (Basta) that is used as a selectable marker gene during tissue culture step. Figures 1, 2, 3 and 4, indicating the restriction maps of these four plasmids (pAB<sub>8</sub>, pBGPH30, pHAL and pNM1, respectively) show the promoters and gene cassette directions.

### B. 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: -

- ❑ Spikes from greenhouse grown plants are collected 10-18 d postanthesis. Immature embryos are isolated and placed with the epiblast exposed on callus induction medium as modified for wheat cell culture. Cultures of immature embryos have been shown to maintain high frequencies of embryogenic and regenerable calli when scutellum growth is maintained (Figure 5a & 5b).
- ❑ After 4-7 days in culture, embryo-derived calli are 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 (Figure 5c).
- ❑ After a recovery period of one week, bombarded calli are transferred to MS selection medium containing 0, 3 or 5 mg/l bialaphos.
- ❑ After four weeks of selection, embryogenic calli (Figure 5d) are 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 (Figure 5e).

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

Transformation experiments resulted in the production of two leaf painting-positive putative transgenics with the rice *HAL2*-like (*RHL*) gene to be tested for both gene presence and expression. No putatively transgenic events were obtained through this season and we expect to have some in winter 2001.

### **C. Molecular analyses of transgenic plants:**

Molecular analyses of  $T_0$  putative transgenic wheat plants started by running PCR to detect the *bar* gene in the two putative transgenics to test for its presence in the wheat genomic background and results were positive (Figure 6). PCR and Southern analysis will be followed to test integration of both the *bar* and rice *HAL2*-like (*RHL*) genes. Stress experiments have started in winter 2001 to test expression of the rice *HAL2*-like (*RHL*) gene. Then later will be imposed by both salinity and by withholding water for different time periods.

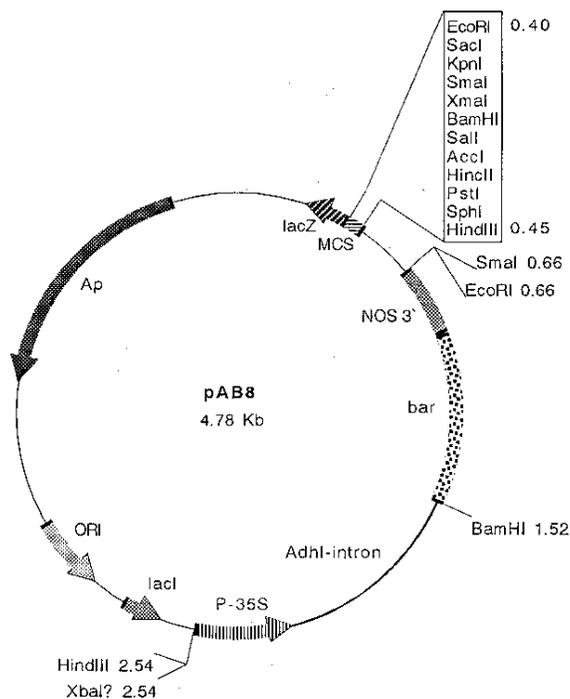


Figure 1. Restriction map of the plasmid pAB<sub>8</sub> with *bar* gene cassette.

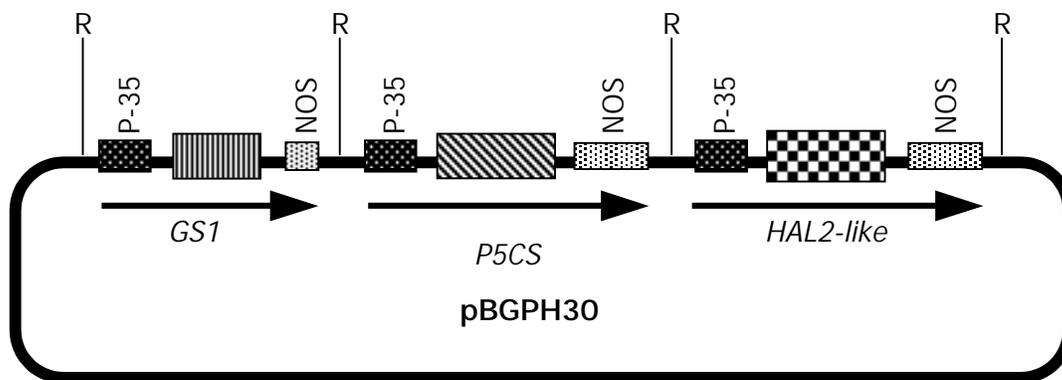
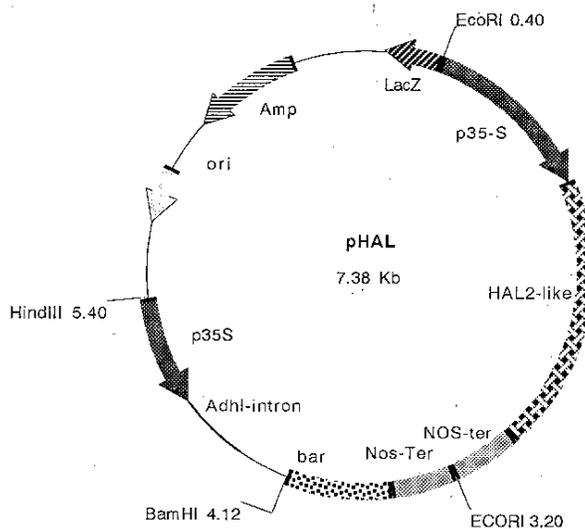
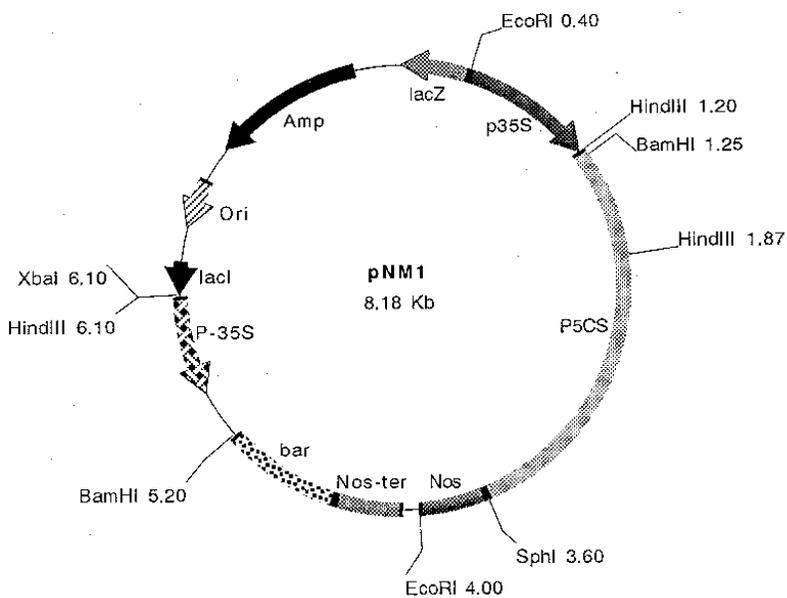


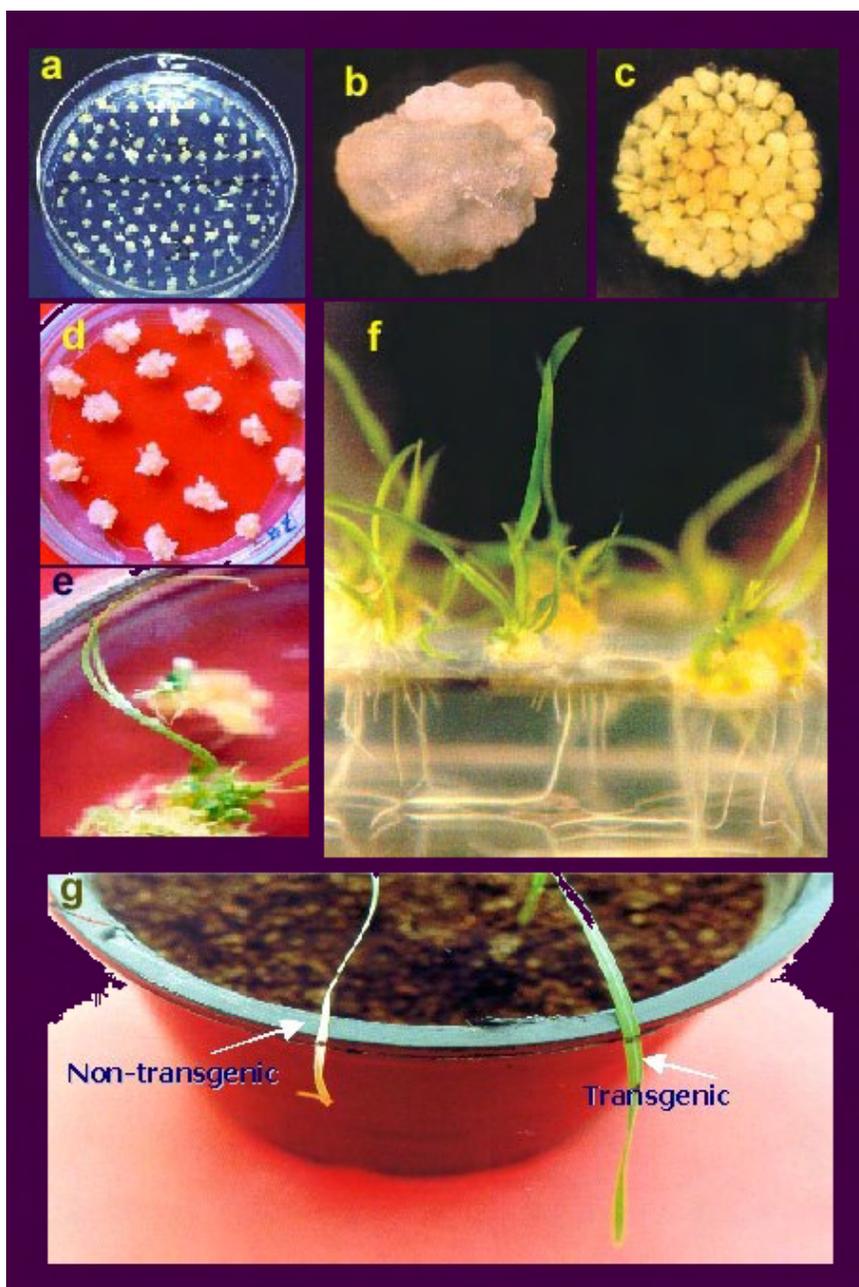
Figure 2. Restriction map of the plasmid pBGPH30 with rice *Hal2-like* gene cassette. (R=EcoRI).



**Figure 3.** Restriction map of the plasmid pHAL with rice *Hal2-like/bar* gene cassettes.

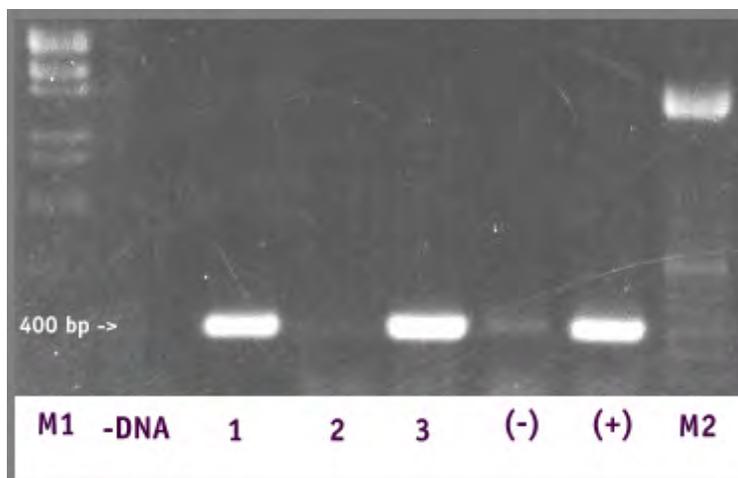


**Figure 4.** Restriction map of the plasmid pNM1 with *P5CS/bar* gene cassettes.



**Figure 5.** Different steps of tissue culture protocol of transformed cells derived from immature embryo.

- a) & b) Culture before bombardment
- c) Osmotic treatment and bombardment
- d) Callus induction
- e) Shoot regeneration
- f) Root formation
- g) Transfer to soil and leaf painting



**Figure 6.** PCR products of partial-length *bar* gene (400 bp) in two putative transgenics (1 and 3) with pHAL plasmid, positive control of plasmid DNA (+), negative control of W.T. plant (-) and no DNA reaction (-DNA). M<sub>1</sub> and M<sub>2</sub> refer to DNA standards of BstEII/λDNA and 100-bp ladder, respectively.

## 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<sub>2</sub> and T<sub>3</sub> seeds will be developed and tested under environmental stress conditions before the process of commercialization. Besides, the novel genes to be isolated at OSU will be patented and commercialized as soon as functions are proven.

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

## Bibliography

- Bahieldin, A.; R. Qu, W.E. Dyer, A.S. Haider and M. Madkour (2000).** A modified procedure for rapid recovery of transgenic wheat plants. *Egyptian J. Genetics and Cytology*, 29: 11-23.
- Boyer, J.S. (1982).** Plant productivity and environment. *Science*, 218: 443-448.

- Chou, I.T.; C.T. Chen and C. Hueikao (1990).** Regulation of proline accumulation in detached rice leaves. *Plant Sci.*, 70: 43-48.
- Csonka, L.N.; S.B. Gelvin, B.W. Goodner, C.S. Orser, D. Siemieniak and J.L. Slightom (1988).** Nucleotide sequence of a mutation in the *proB* gene of *Escherichia coli* that confers proline overproduction and enhanced tolerance to osmotic stress. *Gene*, 64: 199-205.
- Dandekar, A.M. and S.L. Uratsu (1988).** A single base pair change in proline biosynthesis genes causes osmotic stress tolerance. *J. Bact.*, 170: 5943-5945.
- Delauney, A.J.; C.-A.A. Hu, P.B. Kavi Kishor and D.P.S. Verma (1993).** Cloning of ornithine d-aminotransferase cDNA from *Vigna aconitifolia* by trans-complementation in *Escherichia coli* and regulation of proline biosynthesis. *J. Biol. Chem.*, 268: 18673-18678.
- Delauney, A.J. and D.P.S. Verma (1990).** A soybean D1-pyrroline-5-carboxylate reductase cDNA was isolated by functional complementation in *Escherichia coli* and was found to be osmoregulated. *Mol. Gen. Genet.*, 221: 299-305.
- Delauney, A.J. and D.P.S. Verma (1993).** Proline biosynthesis and osmoregulation in plants. *Plant J.*, 4: 215-223.
- Fromm, M.E.; F. Morrish, C. Armstrong, R. Williams, J. Thomas and T.M. Klein (1990).** Inheritance and expression of chimeric genes in the progeny of transgenic wheat plants. *Bio/Technology*, 8: 833-839.
- Glaser, H.; D. Thomas, R. Gaxiola, F. Montrichard, Y. Surdin-Kerjan and R. Serrano (1993).** Salt tolerance and methionine biosynthesis in *Saccharomyces cerevisiae* involve a putative phosphatase gene. *The EMBO J.*, 12: 3105-3110.
- Goas, G.; M. Goas and F. Larher (1982).** Accumulation of free proline and glycine betaine in *Aster tripolium* subjected to a saline shock: A kinetic study related to light period. *Plant. Physiol.*, 55: 383-388.
- Gordon-Kamm, W.I.; T.M. Spencer and M.L. Mangano (1990).** Transformation of wheat cells and regeneration of fertile transgenic plants. *Plant Cell*, 2: 603-618
- Handa, S.; A.K. Handa, P.H. Hasegawa and R.A. Bressan (1986).** Proline accumulation and the adaptation of cultured plant cells to water stress. *Plant Physiol.*, 80: 938-945.
- Hanson, A.D. and W.D. Hitz (1982).** Metabolic responses of mesophytes to plant water deficits. *Ann. Rev. Plant Physiol.*, 33: 163-203.
- Hu, C.-A.A.; A.J. Delauney and D.P.S. Verma (1992).** Osmoregulation in plants: A novel bifunctional enzyme (D1-pyrroline-5-carboxylate synthetase) catalyzes the first two steps in proline biosynthesis. *Proc. Nat. Acad. Sci., USA* 89: 9354-9358
- Kavi Kishor, P.B.; Z. Hong, G.-H. Miao, C.-A.A. Hu and D.P.S. Verma (1995).** Overexpression of D1-pyrroline 5 carboxylate synthetase increases proline production

and helps maintain osmotic potential in transgenic plants during. *Plant Physiol.*, 108: 1387-1394.

**LaRosa, P.C.; D. Rhodes, J.C. Rhodes, R.A. Bressan and L.N. Csonka (1991).** Elevated accumulation of proline in NaCl-adapted tobacco cells is not due to altered D1-pyrroline-5-carboxylate reductase. *Plant Physiol.*, 96: 245-250.

**McCue, K.F. and A.D. Hanson (1990).** Drought and salt tolerance: towards understanding and application. *TIBTECH*, 8: 358-362.

**Murguia, J.R.; J.M. Belles and R. Serrano (1995).** A salt-sensitive 3'(2'),5'-Bisphosphate nucleotidase involved in sulfate activation. *Science*, 267: 232-234.

**Peng, Z. and D.P.S. Verma (1995).** A rice *HAL2*-like gene encodes a Ca<sup>+</sup> sensitive 3'(2'), 5'-diphosphonucleoside 3'(2')-phospho-hydrolase and complements yeast *met22* and *Escherichia coli cysQ* mutations. *J. Biol. Chem.*, 270: 29105-29111.

**Redway, F.A.; V. Vasil, D. Lu and I.K. Vasil (1990).** Identification of callus types for long-term maintenance and regeneration from commercial cultivars of wheat (*Triticum aestivum* L.). *Theor. Appl. Genet.*, 79: 609-617.

**Reggiardo, M.I.; J.L. Arana, L.M. Orsaria, H.R. Permingeat, M.A. Spitteler and R.H. Vallejos (1991).** Transient transformation of wheat tissues by microprojectile bombardment. *Plant Sci.*, 75: 267-243.

**Sivamani, E.; A. Bahieldin, J.M. Wraith, T. Al-Niemi, W.E. Dyer, T.D. Ho and R. Qu (2000).** Improved biomass productivity and water use efficiency under water deficit conditions in transgenic wheat constitutively expressing the barley HVA1 gene. *Plant Sci.*, 155: 1-9.

**Zhang, C.; Q. Lu and D.P.S. Verma (1995).** Removal of feedback inhibition of D1pyrroline 5-carboxylate synthetase, a bifunctional enzyme catalyzing first two steps in proline biosynthesis in plants. *J. Biol. Chem.*, 270: 20491-20496.

# Development of Asian Corn Borer Resistance in Tropical Maize

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## Research Team

### **Pioneer Hi-Bred**

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Dr. Magdy Madkour

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Dr. Ebtissam Hussein

## Overall project goal

Development of *Bt*-transgenic maize technologies for resistance to key insect pests

## Project Importance

Corn Borers (*Sesamia cretica*, *Ostrinia nubilalis*, *Chilo agamemnon*) are serious insect pests in much of the corn growing area of Egypt and are responsible for significant loss of yield. The initial project proposed to introduce into Egyptian commercial corn, (Pioneer proprietary germplasm already tested, registered and grown in Egypt) through available transformation technologies Bt gene(s) which are known to code for proteins that are lethal to lepidopteran species. These genes would be Pioneer proprietary Bt gene(s) and/or those Bt genes belonging to AGERI (i.e., novel Bts contributed by AGERI and discovered as part of this project). Transformed plants and their progeny would be tested for resistance to borer feeding and transgenic events conferring the most resistance to insect damage, and affording the best possible yield and agronomic traits will be identified and selected. Seeds would be registered and sold through Pioneer Hi-Bred through its normal distribution system in Egypt.

## Project background

Maize growing areas in Egypt are infested with three major stem borer insect pests, all of which are lepidopterans. These are *Sesamia cretica*, *Ostrinia nubilalis* and *Chilo agamemnon*, with *S. cretica* causing the most damage. Damage due to *S. cretica* (pink borer) is incurred early in the plant life cycle and results can be catastrophic. The favored oviposition is the second and third

leaves of a plant with extended height range of 20-100cm (Ismail, I., 1989). *S. cretica* infestation is greatest during the period from May to mid June when relatively low infestation levels can produce extensive damage. Larvae feed at the center of the developing plant causing "dead heart disease", which leads to plant death.

There are three generations of *Ostrinia nubilalis* in the Giza region of Egypt (Kira et al., 1975) where corn comes under attack from the early part of May until September (a five month period). In the South Delta region there are more generations, up to six (El-Sadeney, 1965), and the period of attack from *O. nubilalis* is extended. *C. agamemnon* has an economic threshold similar to that of *O. nubilalis* i.e., approximately 20 egg masses/100 plants and eggs are laid at the 5-12 leaf stage. In the U.S., control of *O. nubilalis* has been routinely achieved by externally applied chemical insecticides and biological control agents including *Bacillus thuringiensis*, and in Egypt by the application of insecticides.

With the advent of plant biotechnology have come new tools to control damage from insect pests. Specifically, the introduction of Bt-transgenic maize and other crops during the past few years afforded farmers a means to produce good crops without the use of more traditional and relatively toxic organochemicals for insect pest management. However, these technologies are expensive to develop and have been undertaken in relatively few regions of the world, as yet. Furthermore, the development of transgenic agricultural products depends on sound intellectual property rights (IPR) and regulatory approval policies—the development of which has lagged in many parts of the world. Within the context of the ABSP/USAID program, the project was undertaken to foster the development of these key elements of agricultural biotechnology, especially Egypt.

## Rational for approach

Transgenic corn plants expressing an insecticidal protein derived from *Bacillus thuringiensis*, (Bt) have been field tested and shown to resist insect feeding (Koziel et al, 1993). The insect toxicity of Bt resides in large proteins that have no toxicity to beneficial insects, other animals or humans (Wilcox et al, 1986). Since corn borers feed on corn developmental stages ranging from seedling to maturity, resistance would be most effective if it extended throughout plant growth. The expression of transgenes can be controlled in the plant by putting them under the control of specific promoters (Benfey and Chua, 1989). With the appropriate promoters, expression of Bt genes can be sustained throughout the life of the plant.

Several routes exist to the transformation of corn (see Wilson et al, 1994 for review). The most widely used is particle bombardment of cell cultures or of immature zygotoc embryos (Gordon-Kamm et al, 1990; Koziel et al, 1993, respectively). There is some genotype restriction of lines which can be transformed in this manner. Transgenic maize plants can also be produced by tissue electroporation (D'Halluin et al, 1992). Here again, embryogenic callus is required. Protoplasts have also been used as the starting point for gene delivery (Donn et al, 1990). Here success has been restricted to a single, complex genotype. Genotype restriction can be

addressed either by trying to broaden the range of genotypes which respond to culture (e.g., Armstrong et al, 1990), or through the exploitation of restriction fragment length polymorphism (RFLPs) and/or other molecular markers, to facilitate rapid introgression of transgenes into target genotypes from cultivable material.

There is clearly sufficient precedent described above such that the project was undertaken toward transforming Pioneer commercial corn hybrids (registered and marketed in Egypt) with Bt genes to confer insect resistance is an appropriate, feasible and effective biotechnology approach and one that can be pursued with a high expectation of commercial success.

A strong commitment to research, to quality, and to service have been the guides by which Pioneer has built the strong development, production, marketing and sales of corn, sorghum, sunflower, canola, alfalfa, soybeans, and wheat. Pioneer produces, markets and sells hybrid seed corn, for example, in nearly 100 countries, including through a joint venture, MISR Pioneer Seed Company S.A.E., Nasr City, Cairo, Egypt. (Traditional corn products in Egypt have been of white corn varieties, and the yellow corn market is growing.) Pioneer has a strong interest in the growth and development of improved agricultural biotechnologies that will be essential world wide to meet the needs of the growing world population. Primary plant breeding and research stations are located in 140 locations throughout the world. USAID's ABSP has represented an opportunity for Pioneer Hi-Bred to address a target in a developing country in circumstances of shared cost and, as a consequence, lowered commercial risk. The project offered the chance to explore a clear synergy between the goals of USAID and Pioneer Hi-Bred in the development of a market for insect resistant corn in Egypt.

The expected outcome would be for further strengthening of partnering between industry (Pioneer) and Egypt (AGERI) to develop agricultural biotechnology—to develop Bt technologies for maize with resistance to major lepidopteran pests. It is anticipated that the commercial and agronomic success seen with such *Bt*-maize products in the United States would translate into similar successes in Egyptian agriculture. To succeed would at least require leadership of companies like Pioneer and local education and technology development by institutions such as AGERI.

## Previous Research

AGERI provided a set of *Bt* strains that had been partially characterized for activity versus Egyptian insect pests. Pioneer has completed additional study of those *Bt* materials, but has elected not to pursue them further for control of the maize pests relative to key product market areas.

As has been described in more detail in previous reports, the four visiting scientists from AGERI visited and did research at Pioneer (or for some alternative time at the lab of Dr. Lee Bulla, a collaborator). The experience included hands on work with appropriate technologies for the

project, as well as, introductions to concepts in product development consideration, patenting, intellectual property protection and use, and regulatory work for transgenic materials.

Parallel to the work ongoing in the United States, the senior AGERI members of the project worked to develop maize transformation technologies in Egypt, for Egyptian maize lines.

## Research progress

To summarize for 2000 for this report, there has been generally positive progress, and a few changes to the plans.

First the changes. An extension of the project was arranged to cover the period from September 1999 to Sept 30, 2001. As of January 2000, Terry Meyer has been asked to take on a different role in Pioneer Hi-Bred; with the anticipation (read that as uncertain, but a hope) that he may continue in a consulting capacity to completion of this collaboration.

The four visiting scientists have all been back in AGERI this year, so summaries of their work are included in the reports from AGERI.

The primary research effort to report on Pioneer's behalf regarding this project is that significant progress has been made in characterizing maize plants that have been transformed stably with GUS-reporter constructs of four novel maize promoters. Previous annual reports have been made on these promoters. In summary, transcriptional promoter fragments were isolated from genomic maize DNA. The candidate promoters most of interest to date are referred to as Gos2, Actin-2, Enolase and L41. (Patent applications have been generated as appropriate, with Mohamed Eid Saad listed as a co-inventor.) Clones of the promoters were distributed to AGERI. During my visit of AGERI earlier this year, some nice samples of transiently transformed maize showed interesting gene expression results from these promoters. At Pioneer this year, the stable transformed maize lines with these promoters have been further characterized with regard to relative strength of activity and developmental expression. One or more of them show good promise to serve for expression of *Bt* genes in maize for insect pest control. Slides showing the early results were presented at AGERI during my visit this year.

## Discussion/Implications

Overall, the project is making progress toward the development of materials and methods for making maize transgenic to control insect pests. The visiting scientists involved in this project have worked hard to learn the key technical aspects of such a project. Experience was provided to them regarding technical, legal (intellectual property rights, IPR), regulatory, and team work. As a collective group, the visiting scientists should be able to coordinate at AGERI the experience and lab tools to design experiments for study of maize transgenic for Bt control of insect pests. It is anticipated that Dr. Hanaiya El-Itriby, Dr. Hussein, and Dr. Madkour will

especially guide the next steps of this work at AGERI, with continued discussions with Terry Meyer.

The candidate novel promoters that Mohamed Eid Saad and Mohamed Abdel Wahed worked on need to be further characterized in Egyptian maize lines by AGERI, i.e., in stable transgenic plants to know if they will express transgenes as needed for insect control. Pioneer will also continue characterizing the promoters for use in R&D, and the effort on this overall USAID project has been completed. Once AGERI has the *Bt* gene of choice, the tools and skills should be in place to attain the overall project goal -- generation of *Bt*-maize to study the potential of generating pest-resistant Egyptian maize.

## Highlights of significant achievements

(See also comments for the specific visiting scientists, under *Research Progress*.)

AGERI now has in hand various materials and skills to develop and characterize *Bt*-maize. Gamal continues to work (among other things) on study of *Bts* for maize insect pest control in Egypt. Mohamed Eid Saad knows how to produce cosmid libraries for cloning of novel genes, and has been working on expression constructs to test the novel maize promoters. Mohamed Abd El wahed has completed his PhD, part of which included results of his maize transformation experience at Pioneer Hi-Bred. Hanaiya, Ebtissam, Shireen, Mohamed Eid Saad and the AGERI maize transformation group have had gains in success for maize transformation. Results include transient transformation at AGERI that shows successful gene expression (GUS reporter) driven by the new maize promoters discovered during this study. It should be possible for AGERI to link those promoters to appropriate *Bt* genes or other genes of interest (e.g., selectable markers that may be linked with the *Bt* genes) to test maize plants for insect pest control. With some good luck, maybe one of the new transcriptional promoters will prove to have sufficient activity to be useful, and one or more *Bt* genes may be selected to test in Egyptian maize.

## Publications

Patent applications have been submitted or published regarding: Maize promoters for gene expression. Inventors: Terry EuClaire Meyer, Eric Barbour, and Mohamed Eid Saad, at Pioneer Hi-Bred International, Inc. (Mohamed Eid Saad of AGERI).

## Travel

Terry Meyer, and Catherine Ives traveled to AGERI for a program review in May-June 2000. Terry gave an oral presentation of the research project as part of this meeting, and reviewed data from Pioneer and AGERI's study of the maize transcriptional promoters, and AGERI's transformation efforts.

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## Work Plan 2001

Pioneer Hi-Bred will continue with the transformation and scoring to test the site and relative strength of transcriptional activity from the candidate maize promoters worked on in this project. It is expected that AGERI scientists will likewise work toward testing constructs of these promoters to provide independent assessment of how these materials work under lab conditions at AGERI, and to help the AGERI scientists develop some experience with these materials. Pioneer has discontinued study of the AGERI *Bt* strain(s) and discarded the sample stocks appropriately. It is anticipated that AGERI will continue on internally with screening additional *Bt* strains against key maize pests, and clones of these materials to be characterized for use in controlling maize pests. Pioneer will continue to develop the patent materials regarding the maize transcriptional promoters discovered during this project, and will provide updates on occasion and as appropriate to ABSP and AGERI.

# Maize Transformation for Development of Stem Borer Resistance

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## Research Team (AGERI)

Prof. Magdy Madkour  
Dr. Hanaiya El-Itriby

Prof. Ebtissam Hussein  
Dr. Shireen Assem  
Dr. Gamal Haridy (Entomology)  
Mohamed Abdel-Sadek  
Mohamed Eid Saad

## Project partners

Dr. Terry Meyer, Pioneer Hi-Bred

## Overall project goals and Objectives

- ❑ Establishment of efficient regeneration and transformation system(s) for Egyptian elite maize germplasm.
- ❑ Use the above system(s) to introduce AGERI propriety owned Bt gene(s) to confer resistance to stem borers in commercial maize hybrids.
- ❑ Evaluation of four novel constitutive maize promoters (outcome of Pioneer/AGERI collaboration) in driving the transient and stable expression of the GUS reporter gene.

## 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 agamemnon*) 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 lepidopteron species into Egyptian 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 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 particle bombardment system.

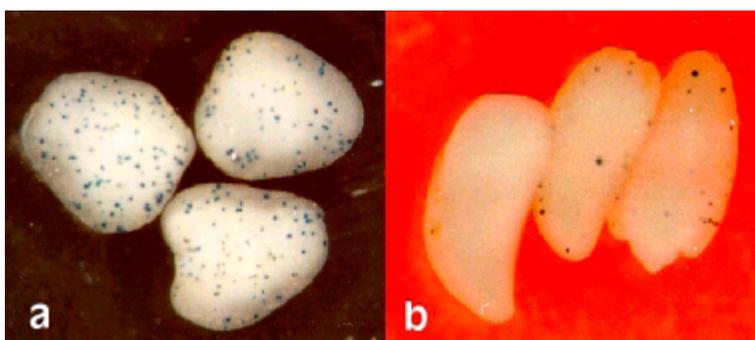
## Previous research

- ❑ Different Egyptian maize inbred lines were screened for their regeneration ability from immature embryo culture & shoot tips using different media.
- ❑ Modification in media composition resulted in improvement of regeneration ability in both systems.
- ❑ Optimum concentration of the selective herbicide agent (Bialaphos) was identified according to the kill curve studies.
- ❑ Transformation was carried out using different bombardment parameters and optimum conditions were identified with both types of explants.
- ❑ Transient and stable expression of GUS gene was detected in transformed calli, shoot clumps and roots respectively.
- ❑ PAT activity was assayed by painting leaves of transformed plants with the herbicide BASTA (1% solution).
- ❑ The integration of transformed genes was confirmed by PCR and Southern blot analyses.
- ❑ The output from one of the training programs at Pioneer resulted in the isolation of four novel constitutive maize promoters (Act-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

- ❑ The four novel constitutive maize promoters with intron and without intron (Act-2, enolase, Gos-2 and L41) have been evaluated for their efficiency in driving the transient expression of the GUS gene in maize immature embryos using the biolistic particle delivery system, in comparison with the ubiquitin promoter as a control.

- ❑ Results based on the transient GUS expression revealed that "gos-2" promoter with intron gave the highest transient GUS gene expression in maize.
- ❑ In an attempt to evaluate the efficiency of the novel promoters in dicot plants. The four novel promoters have been evaluated for their efficiency in driving the transient expression of the GUS gene in tomato leaves using the particle delivery system, results were compared with that of 35S promoter as a control.
- ❑ Results showed that "enolase" with intron gave the highest expression of GUS gene in tomato leaves.
- ❑ Currently plasmid construction is being carried out for constructs that contain these promoters and GUS gene with the bar gene (with or without intron). Their efficiency in stable transformation will be tested.
- ❑ Additional elite maize lines of commercial importance have been screened for their potential in producing embryogenic callus on different media composition.
- ❑ An attempt to improve the embryogenic capability of the highly recalcitrant elite maize line Sd7 has been initiated by crossing this line with the American line A188 known to produce embryogenic calli.



GUS gene expression in immature maize embryos:

a) GUS gene driven by GOS-2 promoter with intron

b) GUS gene driven by GOS-2 promoter without intron

## References

- Kozeil M.G., Beland G.L., Bowman C., Carozzi N.B., Crenshaw R., Crossland L., Dawson J., Desai N., Hill M., Kadwell S., Launis K., Lewis K., Maddox D., McPherson K., Meghji M.R., Merlin E., Rhodes R., Warren G.W., Wright M. and Evola S.V. (1993). Field performance of elite transgenic maize plants expressing an insecticidal protein derived from *Bacillus thuringiensis*. *Bio/Technology* 11:194-200.
- Bohorova N., Zang W., P. Julstrum, S. McLean, B. Luna, R.M. Brito, M.E. Ramos, P. Estanol, M. Pacheco, D. Hoisington (1999). Production of transgenic tropical maize with cry1Ab and cry1Ac genes via microprojectile bombardment of immature embryos. *Theor. Appl. Genet.* 99: 437-444.

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

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## Lead Principal Investigator

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

## Project Partner

Prof. Magdy Madkour, AGERI, Giza, 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

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<sub>1</sub> from the tobacco hornworm (*Manduca sexta*), has been cloned, sequenced and determined to mediate insect toxicity. The principal investigators in insect pests important to Egyptian and American agriculture have also identified several homologues of BT-R<sub>1</sub>. The toxin-binding site of BT-R<sub>1</sub> 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. Ed Grafius 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

Characterization of the expression of BT-R<sub>1</sub> from the tobacco hornworm for the Cry1Ab toxin revealed that the receptor is highly regulated. Developing tobacco hornworm larvae exhibited a significant increase in their level of BT-R<sub>1</sub> mRNA and protein expression as they mature. Furthermore, BT-R<sub>1</sub> expression is highly localized to the midgut of *M. sexta* larvae, which has been shown to be the site of toxin activity. BT-R<sub>1</sub> expression in the midgut increases seven-fold from first through fifth instar with a concomitant decrease (46-fold) in the susceptibility of fifth instar larvae to the Cry1Ab toxin of Bt. Based on these observations, the molecular mode of action of the Cry1Ab toxin, most likely, is mediated through the disruption of the normal physiological function of BT-R<sub>1</sub> and/or additional proteins associated with it. To harm the insect, the toxin must be present in the midgut of *M. sexta* larvae at levels sufficiently high enough to saturate the receptor population and impair normal midgut cell function that is critical to the health and normal development of the hornworm. A manuscript has been submitted to *The Journal of Experimental Biology*.

When BT-R<sub>1</sub> is expressed in artificially cultured *Spodoptera frugiperda* insect cells (Sf21), a significant amount of the protein is produced as a soluble protein in the culture medium. The soluble fraction of BT-R<sub>1</sub> accounts for ~60 percent of the total expressed protein. Soluble BT-R<sub>1</sub> is stable in the culture medium for at least three days at 25°C and for up to seven days at 4°C. This stability will facilitate the accumulation of the protein over an extended period and render it suitable for further molecular characterization. In other words, the expression system in Sf21 cells provides an excellent means for large-scale production and purification of BT-R<sub>1</sub> and its homologues from the tobacco hornworm and the cotton leafworm, respectively. Having large amounts of these receptor molecules will expedite their further characterization. A manuscript has been accepted for publication in *Protein Expression and Purification*.

## Specific Project Objectives

- › Compare proteolytic activity profiles in susceptible and resistant Colorado potato beetle midgut brush border membrane vesicles (BBMV) and gut juice
- › Compare aminopeptidase activities in same
- › Compare stability of Cry3A toxin to BBMV and gut juice proteases
- › Compare toxin binding to BBMV *in vitro* and midgut epithelial cells *in vivo*

## Research Progress

### Proteolytic Activity in Gut Juice and BBMV

Total proteolytic activity associated with the midgut extracts (BBMV and gut juice) from resistant and susceptible strains of the Colorado potato beetle was determined using azocasein as the protein substrate. The reactions were carried out in a slightly acidic reaction mixture (pH 6.0), which is correlated with the physiological pH of the insect midgut. Gut juice from 4<sup>th</sup> instar larval stages of both resistant and susceptible insect strains exhibited similar activity for the hydrolysis of azocasein. Incorporation of reducing agents dithiothreitol or  $\beta$ -mercaptoethanol to the reaction buffer significantly increased the activity in both samples suggesting the presence of cysteine proteinases in the extracts. Incorporation of the protease inhibitors E-64 and leupeptin to the system resulted in ~75% inactivation of the proteolytic activity in both extracts (Table 1). About 25% of the protease activity in the extracts was sensitive to the serine protease inhibitor PMSF. In addition, pepstatin inhibited azocasein hydrolysis by about 20% in the extracts, confirming the presence of aspartate proteinases in the midgut of the beetle.

**Table 1.** Total proteolytic activity in gut juice of fourth instar larvae

Inhibitor	Concentration	Proteolytic activity		Residual activity (%)	
		OD <sub>440</sub> /60min/mg		S	R
None		1.96±0.18	2.00±0.21	100	100
PMSF	5 mM	1.54±0.12	1.52±0.11	78	76
Pepstatin	5 $\mu$ M	1.64±0.13	1.60±0.13	84	80
E-64	400 $\mu$ M	0.47±0.08	0.57±0.09	24	28
Leupeptin	200 $\mu$ M	0.61±0.09	0.66±0.06	26	29

### Activity of cysteine proteases

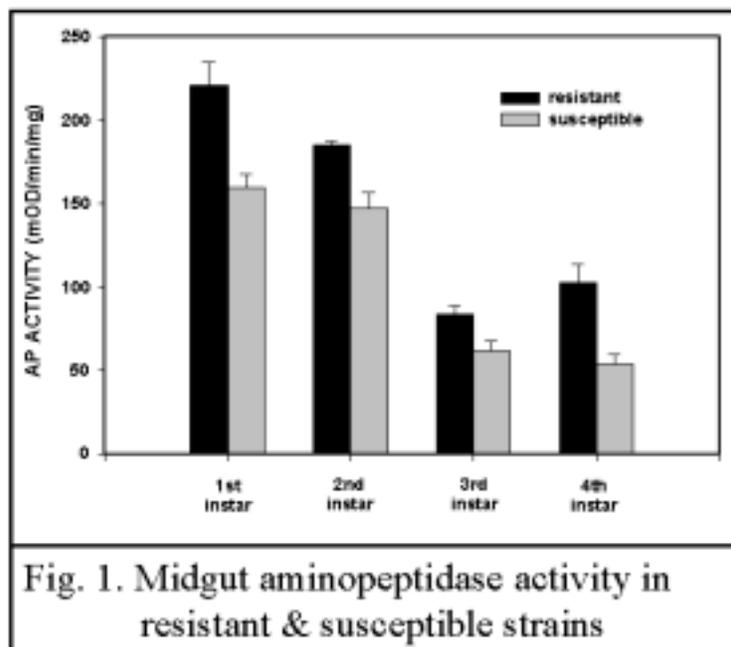
To further characterize and compare the proteolytic activity in the larval gut juice from resistant and susceptible strains, Z-Phe-Arg-MNA was used as a specific substrate for cathepsins B and L, and, Z-Arg-Arg-MNA as the substrate for cathepsin B. No difference was found between resistant and susceptible strains in the proteolytic hydrolysis of these specific substrates. Hydrolysis of the substrates was almost totally inhibited by the inhibitors of cysteine proteases, E-64 and leupeptin, indicating that cysteine proteases represent the vast majority of the activity in the degradation of Z-Phe-Arg-MNA (Table 2). The same results were obtained with Z-Arg-Arg-MNA (data not shown).

**Table 2.** The proteolytic activity in gut juice of fourth instar CPB larvae against Z-Arg-Arg-MNA and Z-Phe-Arg-MNA

Inhibitor	Concentration	Proteolytic activity against Z-Phe-Arg-MNA	
		OD <sub>520</sub> /60min/mg	
		S	R
None		32.3±0.4	33.0±0.5
PMSF	5 mM	32.1±0.2	33.2±0.5
E-64	400 µM	0.5±0.1	0.4±0.1
Leupeptin	200 µM	1.2±0.2	1.2±0.3

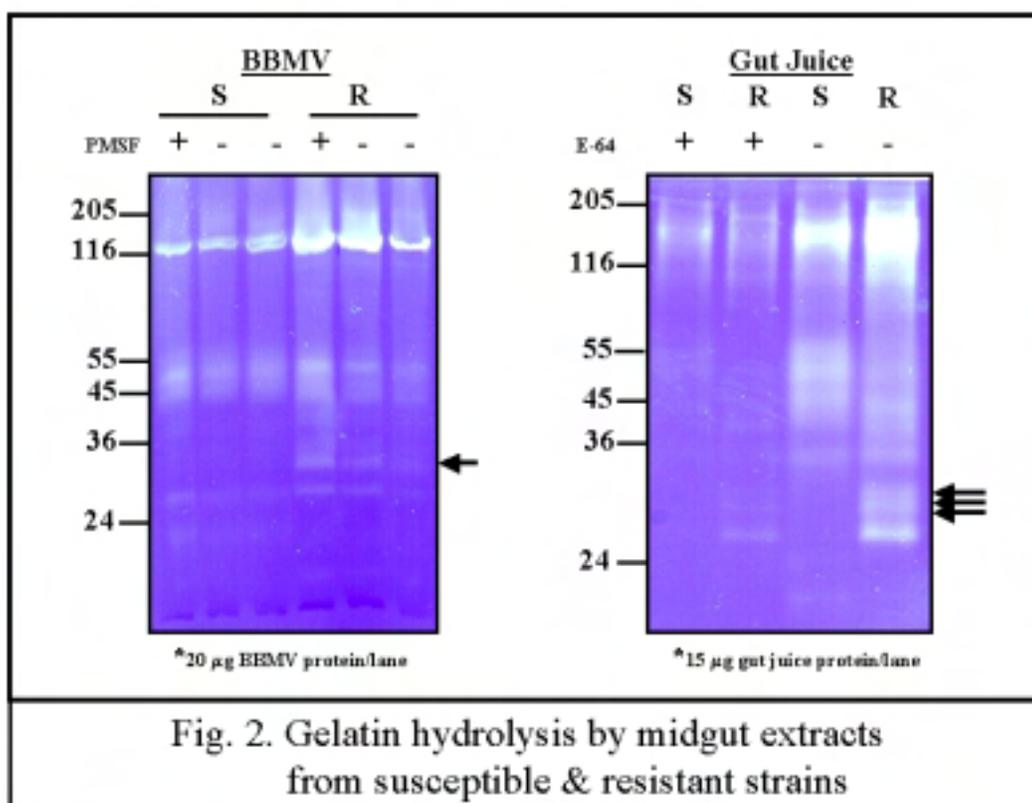
**Aminopeptidase Activity**

In insects, aminopeptidases are found mainly bound to the microvillar membranes of midgut epithelial cells. Indeed, we detected high levels of aminopeptidase activity associated with the BBMV prepared from the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> instar larvae of CPB (Fig. 1). Notably, the resistant beetle strain has higher aminopeptidase activity at all stages of development than the susceptible strain.



### Proteinase Forms in Midgut Extracts

By using zymographic gels containing gelatin as a substrate, qualitative analysis and characterization of digestive proteinases were determined in larval extracts. Electrophoretic separation of larval gut extracts in polyacrylamide gels containing gelatin revealed multiple zones of hydrolytic activity for this substrate. Although this method does not yield an accurate estimation of molecular masses of the proteases because the substrate in the gel matrix interferes with the migration of the proteins, it is a useful method to profile the composition of proteases in the samples. Both the resistant and susceptible strains possess several distinct forms of proteases in the gut juice and in BBMV (Fig. 2).



However, the extracts from the Cry3A-resistant strain exhibited a more complex protease activity profile compared to that of the susceptible strain. Analysis of BBMV and gut juice samples from 4<sup>th</sup> instar larvae of the resistant strain showed additional zones of gelatin hydrolysis on the zymograms, suggesting that the Cry3A-resistant insect may use different enzymes or different isoforms of proteases (Fig. 2). Similar observations were made for BBMV preparations from 1<sup>st</sup> and 2<sup>nd</sup> instar stages of the beetle (data was not shown). Samples also were treated with class-specific irreversible inhibitors of proteases prior to electrophoresis and the proteolytic activity zones on the gels were analyzed for their response to these inhibitors. Almost all forms of the proteases were inhibited by the cysteine proteinase inhibitor E-64 (Fig.

2). There was an obvious activation of proteases associated with the BBMV by PMSF in the gelatin-containing gel (Fig. 2). These results indicate that cysteine proteases constitute a major portion of the gut proteolytic activity in the Colorado potato beetle.

**Cry3A Proteolysis by Gut Juice and BBMV**

To determine whether resistance against Cry3A toxin is related to a difference in the activity of proteases towards hydrolysis of Cry3A toxin, an experiment was performed to compare the proteolysis of this toxin by gut juice and BBMV from the susceptible and resistant strains. Cry3A toxin was incubated with enzyme extracts (1:10, 1:50 and 1:500) and incubated at 37° C for different times. The proteolytic products were analyzed by SDS-PAGE and Western blot analysis. No differences between Cry3A resistant and susceptible strains were observed in these experiments, suggesting that Cry3A toxin processing is not different in resistant and susceptible insects and probably is not associated with the development of resistance against Cry toxins (Fig. 4). Upon incubation with gut juice enzymes, the Cry3A toxin was proteolytically processed, generating a 55-kDa fragment. In the presence of BBMV enzymes, a 38-kDa fragment resistant to further digestion also was formed (Fig. 4). These results suggest that membrane components may be important to the increased susceptibility of Cry3A molecules to proteolysis, perhaps by inducing structural changes on the Cry3A toxin itself.

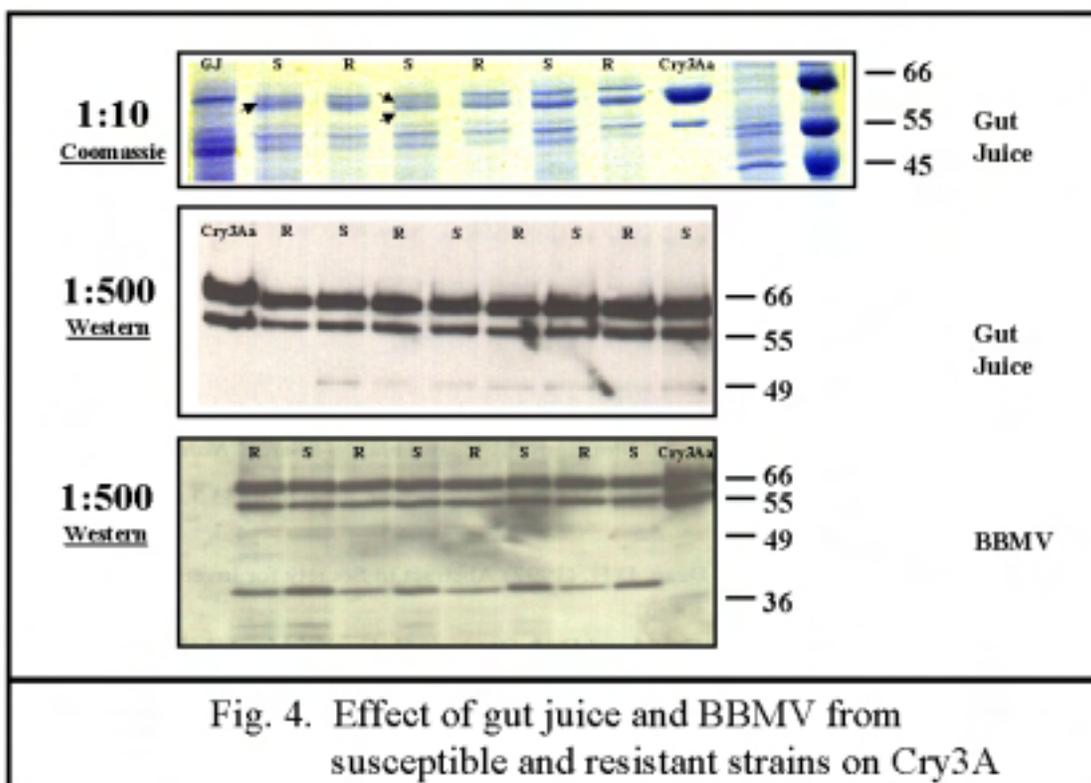
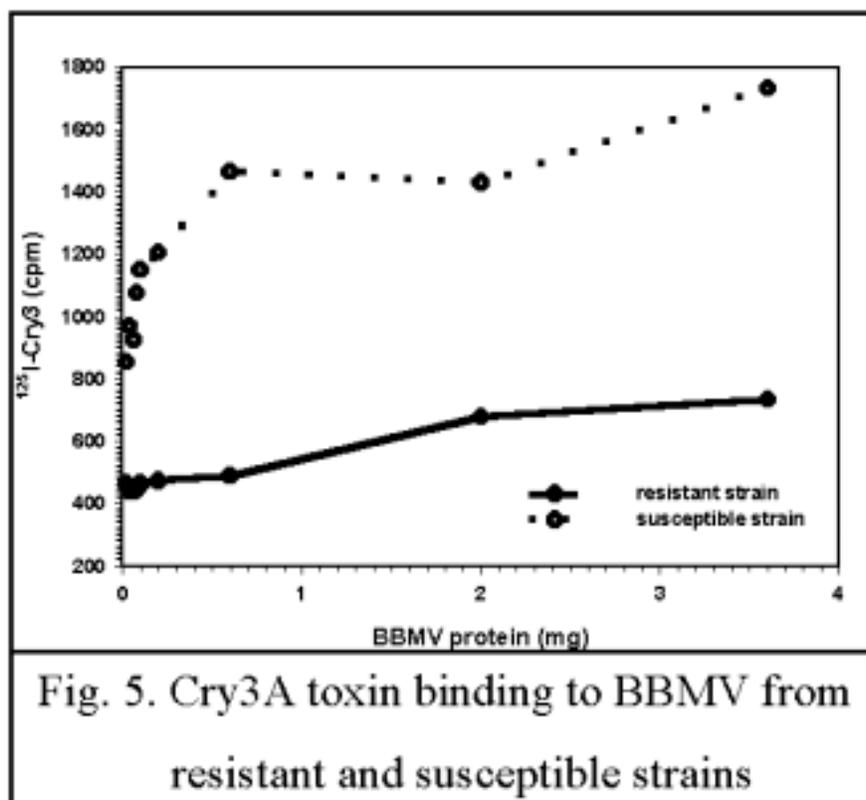


Fig. 4. Effect of gut juice and BBMV from susceptible and resistant strains on Cry3A

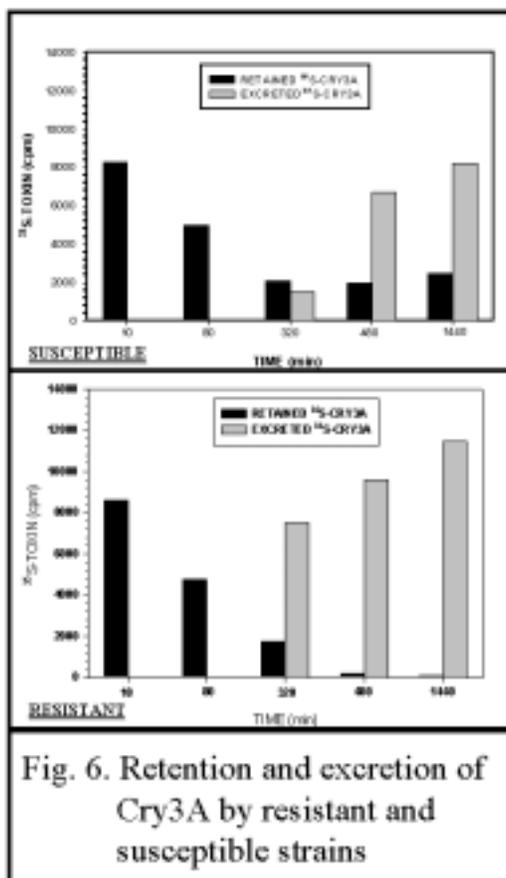
### Ligand binding analysis

To test whether there is a difference between Cry3A resistant and susceptible strains of the beetle in the binding of Cry3A toxin to the gut surface, saturation-binding experiments were performed using  $^{125}\text{I}$ -labeled Cry3A toxin and BBMV preparations from both insects. The results of these experiments showed a significant difference in the toxin binding to BBMV from resistant and susceptible strains (Fig. 5). BBMV preparations from the Cry3A resistant strain bound ~60% less toxin than the Cry3A susceptible strain, suggesting that reduced number of toxin binding sites may be a contributing factor to the resistance mechanism.



### *In vivo* Binding and Excretion

Specific binding of Cry toxins to the apical brush border membrane of midgut cells is a key step in toxicity. To study this event, a force-feeding technique was used that involves the binding of  $^{35}\text{S}$ -Cry3A to the midgut of susceptible and resistant strains of the beetle. It was determined that the susceptible strain retains 10-fold more toxin in its midgut than does the resistant one (Fig. 6) and that the resistant strain excretes 2-fold more toxin in its frass. (Results of Dr. Noah Koller, a collaborator at Michigan State University)



### Effect of Cry3A Toxin on the Midgut of the Colorado potato beetle

The result of toxin action Cry toxins is extensive damage of the midgut epithelial cells. Was investigated, using transmission electron microscopy of midgut cross-sections of the resistant and susceptible strains, the effect of Cry3A toxin on the midguts. The toxin grossly disrupted midgut epithelial cells in the susceptible strain whereas cells in the resistant retained their structural integrity (Fig. 7). (Results of Dr. Leah Bauer, a collaborator at Michigan State University).

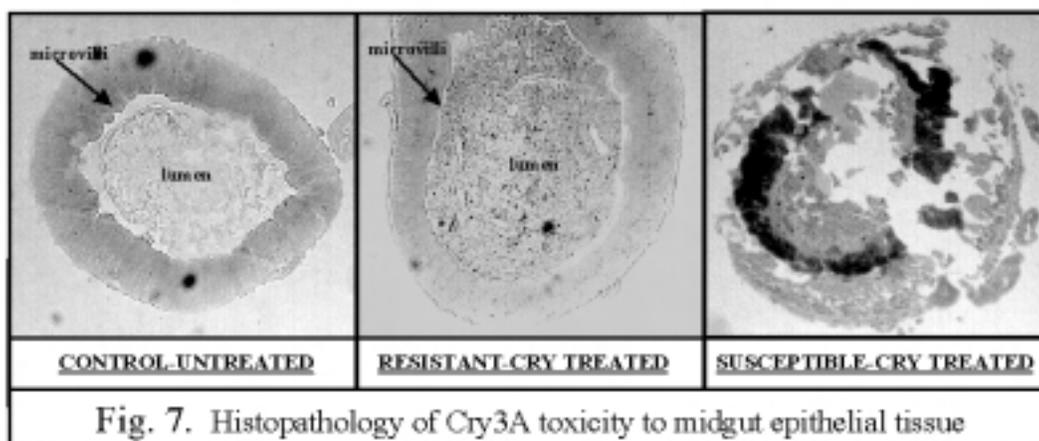


Fig. 7. Histopathology of Cry3A toxicity to midgut epithelial tissue

## Discussion/Implications

The mode of insecticidal activity of Cry toxins involves several steps including solubilization of toxin crystals in the midgut of insects, proteolytic processing and activation of the protoxin molecules, binding of toxins to receptors on the surface of epithelial cells and interaction of the toxin with membrane components leading to disruption of the functional and structural integrity of the apical membrane. Changes in the physiology and biochemistry of the insect gut can alter these processes and diminish the effect of toxin. Indeed, insects that have gained tolerance to Cry toxins have acquired adaptive changes and developed mechanisms that interfere with one or several steps involved in toxin action. In some resistant insects, changes in the proteolytic activity leads to increase in the inactivation of Cry toxins. Conversely, some resistant strains of the Indian meal moth, *P. interpunctella*, lack the enzyme that activates protoxin. In tobacco budworm, *Heliothis virescens*, development of resistance to Cry toxins is correlated with slower processing of protoxin and faster degradation of the active toxin. Overall, reduced activation or increased inactivation of toxin by insect proteases appears to provide a survival advantage when insects are exposed to Cry toxin-containing insecticides. However, in this study, analysis of proteolytic digestion of Cry3A toxin by proteases associated with gut juice and BBMV from resistant and susceptible strains of the Colorado potato beetle did not reveal any difference between the two strains (Fig. 4). So, enzymatic processing or degradation of toxin is not a factor of resistance in this insect. Interestingly, proteases associated with the BBMV preparations from both strains cleaved the Cry3A toxin to generate a specific 38-kDa fragment. This fragment was formed only in the presence of BBMV, but not with gut juice extracts, suggesting that toxin-processing proteases may be located on the membrane or that membrane components might influence the toxin structure, resulting in increased susceptibility to proteolytic attack.

The results presented in this report show that, although there is no quantitative difference in the total proteolytic and cysteine protease activities between resistant and susceptible strains, the resistant strain exhibited a different activity profile of digestive proteases as detected in BBMV

and gut juice extracts (Fig 2, 3). Proteases are important components of pathogen recognition systems and innate immune systems. For example, proteolytic processing is necessary for the activation of the prophenol oxidase cascade and signaling pathways. There is increasing evidences that, in the immune response of mosquitoes to bacteria, the induction of some serine proteases is involved. Presumably, the appearance of some new proteases in the resistant Colorado potato beetle strain may be the result of inducing their expression in response to toxin action.

Interestingly, both the resistant and susceptible strains exhibit an obvious difference in aminopeptidase activity associated with BBMV preparations (Fig. 1). Aminopeptidases are found mainly bound to microvillar membranes of midgut cell. They are the major enzymes in the midgut microvillar membranes of most insects and constitute about 55% of the microvillar proteins in beetles. These enzymes from other insects share common features with mammalian aminopeptidase N (APN) and metallopeptidase of the gluzincins superfamily. Mammalian aminopeptidases N (CD13) as well as other cell-surface peptidases constitute a group of ectoenzymes with a broad functional repertoire, and, they are implicated not only in degradation of terminal peptides and scavenging amino acids, but also in signal transduction by cleaving peptide mediators and modulating their activities.

Currently, there is great interest in innate immunity in insects and mammals. The concept of innate immunity refers to the first-line host defense that serves to create resistance to microbial infections. Three mechanisms contribute to this resistance in vertebrates: (i) phagocytosis of invading microorganisms by blood cells, (ii) proteolytic cascades leading to prophenol activation and melanin formation and (iii) production of antimicrobial peptides. The insect midgut possesses its own immune mechanisms and, like vertebrate epithelia, has specific immune molecules. Membrane aminopeptidases may be implicated in this process because the signal transduction pathways are very similar in vertebrates and invertebrates, suggesting a common ancestral origin for the innate immune system. There is evidence that innate immunity in mouse small intestine may be modulated by aminopeptidase modification of  $\alpha$ -defensins, the antibacterial peptides which have been found in vertebrates invertebrates. Possibly, increasing aminopeptidase activity in the resistant strain of the Colorado potato beetle may be a factor involved in a more effective immune system in this strain compared to the susceptible one, and, it may be involved in the modulation of other responses on the microvillar membrane. Investigation of aminopeptidase genes from *P. interpunctella* strains with different susceptibilities to Cry1A toxin has shown that the aminopeptidase-like mRNA expression levels in the Cry-resistant strain are slightly higher than those in the susceptible strain.

Another possible mechanism of resistance may be caused by decreased affinity by the target protein or receptor. Much evidences exists to indicate that modification in the binding sites for Cry toxins is involved in the resistance mechanism in various insects, just as is the case for the resistant and susceptible Colorado potato beetle strains (Fig. 5). The binding of  $^{125}\text{I}$ -Cry3A to BBMV from the resistant strain was lower than the susceptible one. The low binding is probably due to reduced number or alteration of binding sites in the resistant strain. These results were confirmed the *in vivo* experiments utilizing binding of  $^{35}\text{S}$ -Cry3A to the midguts of the susceptible

and resistant strains. It was determined that the susceptible strain retains 10-fold more toxin in its midgut than the resistant one (Fig. 6). The same time resistant strain excretes 2-fold more toxin in its frass. As a result of this active binding, midgut epithelial cells in the susceptible strain were devastated by Cry toxin whereas resistant cells retained their structural and functional integrity (Fig. 7). The resistant potato beetle was able to escape or recover from toxin action as manifested by decreased toxin binding and increased excretion of toxin compared to the susceptible beetle.

## Highlights of Significant Achievements

Widespread and extensive use of Cry toxin formulations to control insects has increased the likelihood for development of resistance to Bt. Because Cry toxin degradation by proteolysis has been postulated as a possible mechanism for insects to evade deleterious effects of Cry toxin, protease activity profiles were examined as well as toxin-binding in a strain of Colorado potato beetle resistant to the Cry3A toxin of *B. thuringiensis* subsp. *tenebrionis*. Specific proteolytic enzymes were present in midgut extracts and brush border membrane vesicles (BBMV) of the resistant strain that were absent in the susceptible strain. Aminopeptidase activity associated with BBMV from insect midgut was higher in the resistant strain than in the susceptible one. Enzymatic processing or degradation of Cry3A toxin did not differ in these strains and, apparently, is not a factor. However, BBMV from the resistant strain bound approximately 60% less Cry toxin than BBMV from the susceptible strain. Also, saturation kinetics of toxin binding in the susceptible strain is 30-fold greater than in the resistant one. *In vivo* experiments confirm that the susceptible strain retains more toxin in its midgut than does the resistant strain which excretes more toxin than does the susceptible strain. Histological examination revealed that midgut epithelial cells from the susceptible insect are devastated by Bt toxin action whereas cells from the resistant insect retain their structural and functional integrity. Resistance to Bt toxin involves not only decreased toxin binding and increased excretion of toxin but also changes in the composition and activity of midgut proteolytic enzymes, especially elevated aminopeptidase activity. Apparently, midgut aminopeptidase activity is critical to maintaining a protective state, and, together with decreased toxin binding, is involved in innate adaptive responses upon exposure to Cry3A toxin.

## Publications

Expression in *Spodoptera frugiperda* (Sf21) Insect Cells of BT-R<sub>1</sub>, a Cadherin-related Receptor from *Manduca sexta* for *Bacillus thuringiensis* Cry1Ab Toxin. Accepted for publication in *Protein Expression and Purification*.

Developmental and Tissue Specific Expression of BT-R<sub>1</sub> in *Manduca sexta*. Submitted to *Journal of Experimental Biology*.

Insect Resistance to *Bacillus thuringiensis* Insecticidal Toxins. Submitted to *Journal of Insect Biochemistry and Molecular Biology*.

# Technology Transfer and Intellectual Property Rights at AGERI, Egypt

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## Goals

1. Setup an office for 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. Provide awareness and training for all AGERI staff to raise their knowledge in the area of IPR.
4. Provide support documents for technology disclosure, material transfer secrecy information transfer and profit sharing.
5. Provide support for private sector in dealing with AGERI-generated technologies (licensing, grantee technical support and technology upgrading)

## Achievements

### Training program:

Two cycles of training have been concluded as follows:

- › Cycle 1 From Feb-April, 2000
- › Cycle 2 October 15-19, 2000

A total of 40 participants from AGERI had attended the training.

Training materials have been prepared to cover the following subjects:

- › Technology management
- › Intellectual property rights
- › Technology transfer mechanisms
- › Protection of intellectual property rights
- › License agreements
- › Materials transfer agreements

- › Technology disclosure
- › IPR policy
- › Role of technology transfer office

**Training program for other ARC staff:**

According to the request of the Food Technology Research Institute, the OTTIP run a training program for 8 of their senior staff covering the subjects mentioned above.

**Patent applications:**

Three patent applications have been filled with the Egyptian Patent Office for technologies developed within AGERI and with AGERI staff as follows:

1. *Chitinase gene with antifungal and insecticidal activities*: R. Anan et al, application # 2000-7-893
2. *Novel Egyptian dehydrin gene (VMDHN10)*: A.Nada et al, application # 2000-9-1152
3. *Novel maize promoters*: M. Saad et al, application # 2000-7-894. The application has been abandoned after the one year-period allowed by law.

**Brochures:**

An Arabic brochure dealing with the issue of “Intellectual Property Rights” has been compiled from different sources. It had been edited and formatted for printing

**IPR activities:**

The office have been involved in all activities concerning IPR issue for AGERI (Upon requests from the Director of AGERI).

- Numerous Material Transfer Agreements have been issued with different institutions around the world (USA, France, etc).
- Negotiation with some private companies to licensing some AGERI technologies and/or cooperation in research programs.

**IPR policy for ARC**

The office has been actively participating in preparing the Internal Policy for Intellectual Property and Technology Commercialization of Agricultural Research Center. The policy required formal approval from the Minister of Agriculture to setup the office for ARC.

**Furniture, hardware and software for AGERI Office:**

All furniture, hardware and software required for full scale running OTTIP office for AGERI had been ordered and bought. The new location for the office is in the final preparation stage within this fiscal year.

# Southern African Regional Biosafety Program

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## Principal Investigator

J A Brink, ARC-Roodeplaat, South Africa

## Project partners

M Koch, Innovation Biotechnology, South Africa

## Reporting period

October 2000 – February 2001

## 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 Cartagena 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 Cartagena 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 raise new funding and its activities ceased. The current status of biosafety in the SADC region as well as Africa are summarised in Addendum 4, 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:

- ◆ Heartwater livestock vaccine (Zimbabwe, donor supported)
- ◆ Insect-resistant cotton (Zambia, private sector)
- ◆ 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 that 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 Cartagena 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 ½ day 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 6 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

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

- ✓ A total of seven core target countries were identified for the SARB program.
- ✓ The governments of target countries were requested to nominate at least 3 delegates per country to be part of the official SARB working group (see list of current working group members in Addendum 1).
- ✓ The first working group meeting was held in Pretoria, South Africa on 19 and 20 November 2000 (see Activity report on working group meeting in Addendum 2)

**Activity 2 Regional Workshop on Biosafety:**

- ✓ 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 complex of the Agricultural Research Council in Pretoria, South Africa.
- ✓ A key success factor for Activity 2 is 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 was visited during the period 28 January to 28 February 2001 (see short summaries of visits in Addendum 3) 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 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 and the specific protocol arrangements with respect to communication and nomination of delegates are clearer.
- ✓ Some country delegations for the Regional Biosafety Workshop have already been nominated. The deadline of 28 February for receiving nominations will have to be extended to allow a country such as Tanzania to nominate appropriate delegates.

#### Activity 4: Journalists/Media Course:

- ✓ Preparations for this activity started in January 2001. This activity will be 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 will compliment the USDA course and it will allow the media representatives from the core target countries to directly interact with scientists and policy makers. The week of 21 May has been scheduled for these 2 activities. This will also coincide with the CGIAR mid term meeting to be held in Durban, South Africa.

## Travel of project personnel/partners

Project Personnel	Country, City	Period
Dr Brink and Ms Koch	Botswana, Gaborone	28 – 30 January 2001
Ms Koch	Namibia, Windhoek	31 January 2001
Dr Brink and Ms Koch	Zimbabwe, Harare	4 – 6 February 2001
Dr Brink and Ms Koch	Mozambique, Maputo	8 – 9 February 2001
Dr Brink	Malawi, Lilongwe	12 – 14 February 2001
Ms Koch	Mauritius	16 – 20 February 2001
Dr Brink	Tanzania, Dar-es Salaam	25 – 28 February 2001
Ms Koch	Zambia, Lusaka	25 – 27 February 2001

Project Partners	Institution	Period
Dr J Lewis	USAID Washington	20 – 22 November 2000
Dr C Ives	ABSP, MSU	20 – 22 November 2000

**APPENDIX 1: SARB Working Group**

<b>Name</b>	<b>Address</b>	<b>Contact numbers</b>
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## Appendix 2

# SARB Program, Activity Report 1

## -- Establishment of a Regional Working Group --

### **Aim**

The first activity of the Southern Africa Regional Biosafety program (SARB) was to establish a regional biosafety working group and organise a meeting of the working group. The meeting was a workshop to identify regional needs, review the SARB proposal and assess its ability to meet local needs. In addition, the working group members from each of the six core countries were requested to draft a mini-business plan on national projects that could form part of the SARB program. These national proposals were presented to the group.

### **Attendance and Activities**

A total of sixteen delegates were invited from the six core countries. One Zambian and one South African did not turn up, while one South African sent a replacement. The delegates were representatives of existing (Mauritius, Zimbabwe and South Africa), developing (Malawi and Zambia) or still-to-be-initiated (Mozambique) biosafety frameworks. This ensured good input and the quality of delegate participation was focused and high.

The delegates were informed of the management structure of the SARB program, the funding sources and the proposed activities. Regional needs, the scope to the program and their relationships to other biosafety capacity building initiatives were thoroughly discussed. Suggestions were made on how to modify the program to better address current needs. Countries then responded to a general questionnaire on the status of biosafety and developed draft national proposals for projects that could be funded by the second phase of the project.

### **Outcome**

Once the delegates questions were answered about the background of the SARB program, how it was developed and how it would fit into other biosafety capacity building initiatives, they were unanimous in their approval of the program and it's potential benefit for the region.

### **Next step**

All delegates have returned to their countries to consult internally on the SARB program, encourage participation in the activities, identify suitable candidates for forth coming regional activities and further develop their national project proposal for in-country activities in the second year.

*Johan Brink and Muffy Koch*  
November 2000

### Appendix 3

## Interim report on the SADC country visits for the SARB program

### Botswana

This visit enabled meetings with the following people:

- Dept of Agric, Strategy and Planning
- The Director, Agricultural Research Services (ARS)
- 11 scientists with the ARS
- Scott Alan at the RCSA USASID office
- The Director of the National Conservation Agency and
- The deputy attorney general
- 

Overall, the response to the SARB program was positive. There was disappointment that Botswana was not identified as a target county and this country has not yet applied to UNEP for funding to establish a biosafety framework. However, the GBDI meeting in Sept. 2000 led to the formation of an informal working group to drive biosafety development. This Working group includes all the key players and has established the critical inter-sector relationships that will be necessary for establishing a national biosafety framework.

It was clear from the meeting with ecologists, entomologists, plant pathologists and agronomists that these scientists are keen to be trained in biosafety risk assessment and risk management.

### Namibia

This meeting served two functions - to invite Namibia to join the SARB program and to invite them to join the group of core counties for the program. Namibia has an established Biosafety Alliance and may of these members where at the meeting. In addition, there was a member of AFMA (Animal Feed Manufacturers' Association).

The presentation raised many questions and there was a lot of discussion. Clarity was sought on how this project would integrate into UNEP activities on the continent and what influence the US would exert on the training and the format of decision-making processes. The project was well received and the group used the remaining meeting time to discuss Namibia's involvement. They clarified the process to follow to formalize their involvement (an invitation to the Minister with copies to other strategic role-players) and provided names for the invitations for the

### Zimbabwe

This visit started with a meeting with two members of the working group and it was clear that considerable effort had been put into their preparation for the visit and their SARB involvement. The primary biosafety needs identified by the working group fall into two clear categories: public awareness and scientific risk assessment training.

A further 4 visits enabled us to meet with members of the Zimbabwe Biosafety Board, who advise on all applications for work with GMOs. All of these meetings indicated support for the SARB project and willingness for strong participation from Zimbabwe. In addition, discussions with ZIMBAC (the Zimbabwe Biotechnology Action Council) suggest that they may have

secured funding for extensive public awareness on biotechnology and, if so, this could enable the SARB project to focus its funding on scientific risk assessment training.

Zimbabwe is running its first field trial this year, but has had an animal vaccine trial underway for since last year. Mr Abisai Mafa, the Registrar of the biosafety legislation, hosted our visit. The meetings appear to have raised his standing in the eyes of the Biosafety Board and he was visibly more confident at the end of the visit.

### **Mozambique**

The visit to Mozambique was coordinated by the one active working group member – Anabela Zacarias from the National Agriculture Research Institute. She arranged meeting with the following people:

- The deputy director of her institute (NIRA)
- The deputy director of the Department of the Environment and
- An officer in the national Department of Agriculture.

These meetings enabled us to sensitize government officials to the SARB project and to gauge their interest in the proposed activities.

It is clear that the two working group members from the Dept of Environment are not able to assist Anabela with the project: Jose Halafo has been moved to focus on fisheries and Jaime Muchanga is in the information office (library). The deputy director of NIRA agreed to find additional support for Anabela and to inform us of how to request an additional, active working group member from the department of the environment.

The deputy director in the Department of the Environment was well versed in the current status of biosafety under the CPB and sees the SARB project as a valuable means for his department to address their responsibilities in response to the CPB. He is acutely aware that Mozambique lies geographically downstream from all its neighbouring countries and that whatever happens regionally with GMOs could impact on Mozambique. The Department of Agriculture officer was too junior to make any input into the project, but was taxed to brief the deputy director of our discussions and the input needed for the March 2001 meeting.

Mozambique is some way behind the other target countries with respect to biosafety. However, interest is high and the opportunities for agriculture are enormous. There will need to be some additional stimulus to start field trials in this country, where issues of floods, food security and infrastructure dominate all agricultural planning. Some organisation or company will need to request trials or a joint GM project to give grounds for concrete action in this country.

### **Zambia: To follow**

**Malawi**

The visit to Malawi was co-ordinated by D Harvey Kabwazi, a SARB working group member. Dr Kabwazi is also the Chairman of the Biosafety Commission in Malawi. The first meeting of this visit was scheduled with the Deputy Director of Environmental Affairs. This department coordinates Biosafety issues in Malawi. This was followed by a meeting with the SARB working group attended by Dr Kabwazi and Mr. Saliifu. Dr Imbali and Ms Yanira could not attend this meeting. It was suggested that Mr. Salifu be co-opted as member of the working group.

A meeting was also scheduled with the Deputy Director, Technical Services of the Dept. of Agriculture.

A round table discussion was also scheduled with some of the members of the Biosafety Commission of Malawi. The SARB program was presented to this group and questions with respect to the official involvement of SADC was raised at this meeting. After this meeting a courtesy visit was made to the Director of the Environmental Affairs Dept. This dept. pledged its full support for the SARB program.

**Tanzania**

After initial difficulties with arranging meetings with the relevant biosafety officials in Dar-es-Salaam, a meeting was scheduled at the Tanzania Commission for Science and Technology (COSTECH). This meeting was attended by 16 officials from various government departments (COSTECH, Dept. of Agriculture, Vice-Presidents Office-Div. Of Environment, National Environment Management Council, Tanzania Bureau of Standards, and Universities). The Director General of COSTECH chaired the meeting. A presentation was made on the SARB program followed by some enthusiastic discussions and comments. The main focus of this discussion was the exclusion of Tanzania from the core target country selection. The conclusion of this meeting is that an active interest in Biosafety exists in Tanzania, the various government departments communicate well with each other and seems to be well organized, Tanzania is way ahead with respect to biosafety compared to its immediate neighbors, Tanzania participates actively in the Bio-Earn project and Tanzania will request their inclusion as a core target country.

Follow-up meetings were also arranged with the Director, Natural Resources of the National Environment Management Council, the Director General of the National Institute for Medical Research, the Director General Tanzania Bureau of Standards, the University of Dar-Es-Salaam (Botany Dept.), the Ministry of Agriculture (Mikocheni Agricultural Research Institute), the Director Business Registration and Licensing Authority, the Director of Science and Technology of the Ministry of Science and Technology and Higher education.

**Mauritius**

This visit gave the local biotechnology research community an opportunity to raise their concerns about the delays with the biosafety legislation with the Permanent Secretary of Agriculture. There are many ways the SARB funding can be used to address the immediate constraints and this was a good entry point for discussion about the projects and its potential value for the island.

Additional meetings with the MSIRI (sugarcane research institute) highlighted their needs with regard to field trials of GM cane and input into the future of agriculture on the island and their continued role in SADC as an agricultural research institute. The planned meeting with the department of the environment didn't happen (they failed to arrive), but the documentation and input requests were left with the department for a response.

It seems likely that the SARB project will encourage the MSIRI to apply for a field trial within the accepted Guidelines on Biosafety within 2001. This will necessitate a response from the Permanent Secretary and could stimulate progress on the Act and initiate public awareness. All of these activities could form part of the in-country SARB-funded projects.

### **Summary**

The suggestion to visit the SAC countries at the outset of the program was a very good one. This gave the coordinators a chance to:

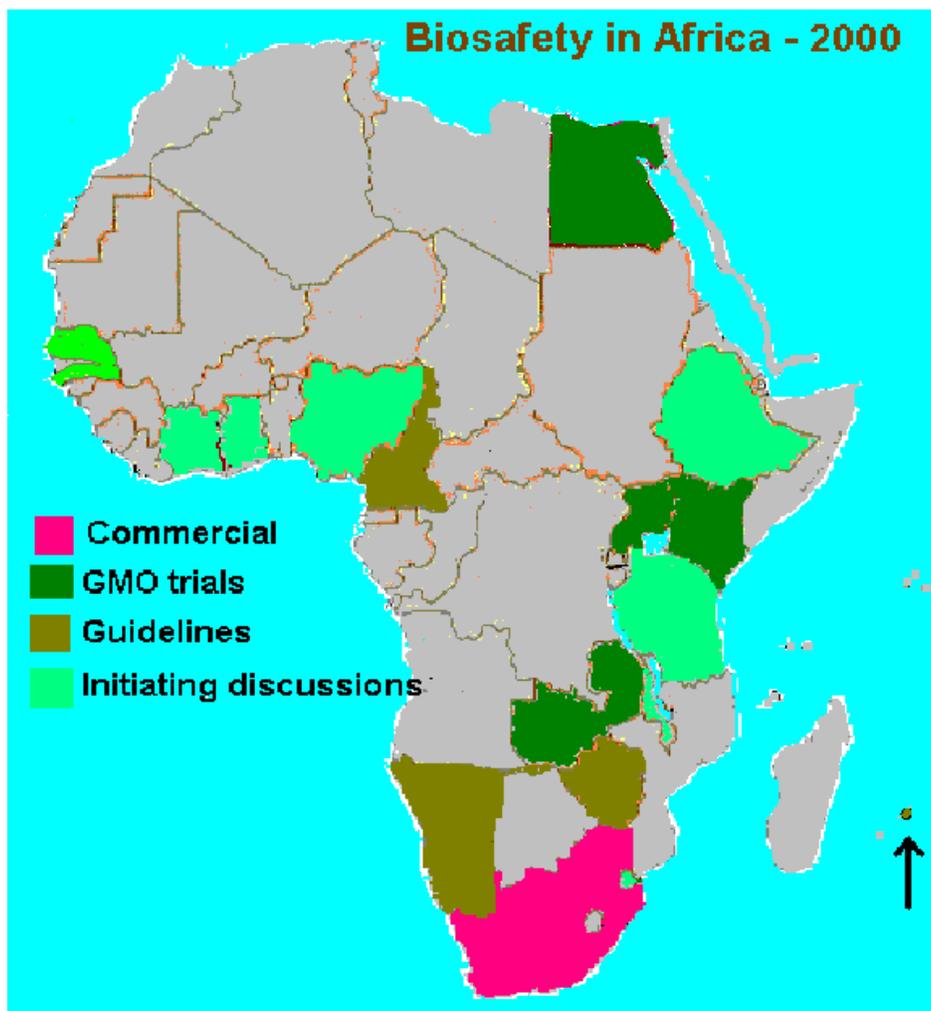
- follow up with the working group members of the target countries,
- get a first hand understanding of the status of biosafety and the critical role players in each country and
- ensure that invitations for the regional meeting in March 2001 were delivered to the correct stakeholders.

The visits also encouraged working group members to follow up on their assignments and to make contact with the relevant stakeholders in their country. From these first visits it is clear that Botswana, Namibia, Tanzania and Zimbabwe will be sending strong delegations to the March meeting and that there is strong support for a regional biosafety initiative from all the countries visited.

*Muffy Koch and Johan Brink*  
28 February 2001

### Appendix 4

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



# Biosafety Activities Under the ABSP Project

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## Principal Investigator

Dr. Patricia L. Traynor, Virginia Polytechnic Institute and State University

## Project Objectives with Benchmarks

The ABSP biosafety project has two components. The first is *Short-Term Technical Assistance*, which may include but is not limited to the following tasks:

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

The second component is the development of a *Biosafety Review Workbook*, which is to be used in conjunction with a program of in-country training workshops. The workbook (and course) will contain an overview of the context for biosafety review, risk assessment, risk management, monitoring, risk communication, and six (to start) case studies to teach participants how to conduct biosafety reviews that lead to decision-making.

## Research Progress

### **Progress in Short Term Technical Assistance:**

- › Presentation on “Biotechnology and Biosafety: Issues, Non-Issues, and Hidden Concerns,” Michigan State University, Michigan
- › Presentation on “Commercializing Agricultural Biotechnology Products in Egypt: Analysis of Biosafety Procedures,” Cairo, Egypt
- › Advisor to ASARECA Working Group at September meeting to discuss options for biosafety implementation within member countries

### **Progress on the Biosafety Review Workbook:**

- › Authors identified for all topics

- › First drafts of material for all sections received
- › First drafts of six case studies received
- › First combined draft circulated to all authors for comment
- › Second combined draft in preparation

## Travel

The following project travel was undertaken in 2000:

- › April 19-21 to MSU; present seminar and consult with ABSP staff
- › May 26 - June 1 to Cairo, Egypt; attend CUB Symposium, give presentation
- › Sept 1-9 to Entebbe, Uganda; attend meeting of ASARECA Committee of Directors

## Work plan

The following tasks are scheduled from January 1 - August 31, 2001 under the terms of the existing three-year contract for 20% time commitment:

- › A final draft of the biosafety workbook will be distributed to selected reviewers; their comments and input will be used to revise the book as necessary.
- › The final text and supplemental materials for the workbook will be completed and ready to publish.
- › Other support activities will be continued as in previous years:
  - Attend workshops and conferences, as requested.
  - Prepare talks and presentation materials.
  - Consult with scientists and government officials, as requested.
  - Review biosafety documents from USAID and partner countries, as requested.

## *AgBiotechNet – CABI Publishing*

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### Principal contacts

#### **CABI Publishing, CAB International, UK**

Liz Walker, 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.

### 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 2000 include:

- ◆ **Improving phosphate acquisition efficiency in transgenic plants by citrate overproduction** (José López-Bucio, Verenice Ramírez-Rodríguez and Luis Herrera-Estrella) ABN 058
- ◆ **Labelling GMOs in food: Trojan horse or good policy?** (Julie A. Caswell) ABN 059
- ◆ **Will global agbiotech pay? a review of sales trends** (Richard Leech) ABN 054
- ◆ **GMOs in developing countries** (Alain Weil) ABN 052
- ◆ **The concept of substantial equivalence in safety assessment of foods derived from genetically modified organisms** (Marianna Schauzu) ABN 044
- ◆ **MIRCEN networking: capacity-building and BNF technology transfer in Africa and Latin America** (Nancy Karanja, J. Freire, M. Gueye and E. DaSilva) ABN 043
- ◆ **Could agricultural biotechnology contribute to poverty alleviation?** (Charles Spillane) ABN 042
- ◆ **Genetically modified plants: developing countries and the public acceptance debate** (John H. Skerritt) ABN 040
- ◆ **Transgenic resistance to rice yellow mottle virus disease: highlighting some of the issues facing the potential introduction of biotechnological products into Africa** (Yvonne M. Pinto) ABN 039

**Books** – full text of key titles – with more to be added shortly.

The **Links**, **Patents**, **Topics**, and **Calendar** sections are all updated on a regular basis and provide comprehensive reference points for existing and new users.

The **Links** section now includes a feature which allows users to incorporate a link to **AgBiotechNet** on their website. The feature, called Link Exchange, also allows the user to email CABI with the details of their website so that we can create a reciprocal link to their site.

The **Calendar** section of **AgBiotechNet** is one of the most comprehensive listings of relevant events etc. in this subject area. The service will, in due course, also provide a linkage function to conference proceedings due to be published elsewhere.

**Conferences** – Important new papers from conferences such as Public Awareness and Risk Assessment in Agricultural Biotechnology (key papers from seminar/workshops held in Buenos Aires, Argentina and Santiago, Chile, August/September, 1999) continue to appear on the site.

**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 over 80,000 records available on the database, with around 1300 added per month – a major increase over 1999 levels. The search interface will be significantly enhanced in March.

**ListServ** -- AgBiotechNet has a listserv facility which alerts users to new developments e.g. publication of new review articles etc. The number of registered listserv members has grown to over 370 since the launch of the service.

**AgBio Jobs** – this very popular feature covers jobs throughout the world and individual jobs attract hundreds of viewings. The Editor moderates submissions. Access to the service and submission of positions is free of charge.

## Access to AgBiotechNet

For the year 2000, access to **AgBiotechNet** was available on the following basis:

1. As a paid-for electronic subscription product available from *CABI Publishing*;
2. As an added value feature of a subscription to the printed journal *AgBiotech News and Information* published by *CABI Publishing*;
3. To our partners in **AgBiotechNet** including the ABSP staff and its grantees;

In 2001, we are introducing an individual rate, which represents a substantial discount on the standard institutional rate.

## Visiting countries

Argentina	Jamaica	Thailand
Belarus	Jordan	Tonga
Bolivia	Kazakhstan	Trinidad and Tobago
Botswana	Kenya	Turkey
Brazil	Korea (South)	Turks and Caicos Islands
Bulgaria	Latvia	Ukraine
Cambodia	Lithuania	Uruguay
Chile	Macedonia	USSR (former)
China	Malaysia	Mauritius
Colombia	Moldova	Venezuela
Costa Rica	Mongolia	Yemen
Cote D'Ivoire	Namibia	Yugoslavia
Croatia	Nepal	Zambia
Cuba	Nicaragua	Zimbabwe
Czech Republic	Pakistan	
Djibouti	Peru	
Ecuador	Philippines	
Egypt	Poland	
Estonia	Qatar	
Fiji	Romania	
Georgia	Russian Federation	
Ghana	Singapore	
Guatemala	Slovak Republic	
Guyana	Slovenia	
Hungary	South Africa	
India	Sri Lanka	
Indonesia	Taiwan	
Israel	Tanzania	

**Subscriber registrations:** 371 institutions and individuals in the following countries

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

## Developing Countries

CABI has established a relationship with the International Service for the Acquisition of Agri-Biotech Applications. This will extend access to another 20 developing country institutions. CABI has used the skills acquired in constructing *AgBiotechNet* in a capacity-building initiative that involved training the Managing Editor of ISAAA Knowledge Center for Crop Biotech, and representatives of Biotechnology Information Centers around the developing world. The agreement we have with them requires news on biotech from developing countries to be supplied 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 entirely free service 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 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

Conversations with individual developing country users indicate a high degree of satisfaction. However, *CABI Publishing* is planning a comprehensive survey of users and potential users in early 2001 to establish opinions on existing features and some new features planned.

## The Year Ahead

**AgBiotechNet** is transferring its operation from a flat-file system to a database-driven version. The advantages of this include a major reduction in admin time, the ability to personalise features to allow users to get straight to the material they want, and to generate specific email newsletters for individuals, which will be a major advantage for those with limited connection time.

We anticipate that our relationship with ISAAA and other developing-country focused biotech organizations will continue, and **AgBiotechNet** will continue to be seen as a core essential resource for those who want the latest information on agricultural biotechnology, and especially those concerned with impacts on developing countries.

CABI is committed in its Information for Development strategy to develop further initiatives relating to GMOs and biosafety, relating to developing countries. This will make use of the core capacity CABI has in its Bioscience, Publishing and Information for Development strands, and rely on our network of Member Countries and international centres.

## Conclusions

The contents and user community of **AgBiotechNet** have continued to grow since launch in January 1999, and are starting to involve novel ways of tailoring it 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. Discussions with ISAAA have been fruitful, leading to an extension in access and the depth of coverage of developing world issues. We are continuing to discuss consortium arrangements that can expand access at substantial discounts or free to end-users.

## AgBiotechNet – Usage Statistics

