AGRICULTURAL BIOTECHNOLOGY SUPPORT PROJECT

EGYPT PROJECT FINAL REPORT

October 15, 1995 – September 30, 2002

Supported by the United States Agency for International Development (USAID) and implemented by Michigan State University under Egypt Grant: 263-0240-G-00-6014-00

Submitted by:

Dr Johan Brink

Director ABSP

Michigan State University

East Lansing, MI 48824

Tel: (517) 432-1641 Fax: (517) 353-1888

Email: brinkj@msu.edu

All information pertaining to the management, research and financial activities of the Agricultural Biotechnology Support Project are confidential and proprietary to Michigan State University and other contributing institutions and agencies. No use can be made of this information without the express written permission of the appropriate institution.

Table of Contents

Introduction

Project Background

The Agricultural Biotechnology Support Project (ABSP) at Michigan State University has collaborated with Egypt since October 1992 under the Commercialization and Utilization of Biotechnology (CUB) Project, funded by USAID/Cairo. Phase I of the project, which involved the establishment of the Agricultural Genetic Engineering Research Institute (AGERI) was funded under the former National Agricultural Research Project (NARP). Phase I began in early 1993 and ended in September 1995. When the NARP project was phased out, the ABSP collaboration with AGERI then came under the new Agricultural Technology Utilization and Transfer Project (ATUT), from which Phase II, Commercialization and Utilization of Biotechnology (CUB) is funded.

ABSP's main collaborator in this project has been the Agricultural Genetic Engineering Research Institute (AGERI), part of the Egyptian Agricultural Research Center (ARC). AGERI was jointly established by the Egyptian government and the United Nations Development Program in 1989, with the aim of using genetic engineering to develop crops with enhanced traits, such as disease and insect resistance and salt and drought tolerance. A high level of support from the Egyptian government for biotechnology research has enabled AGERI to develop state-of-the-art laboratories, presenting an ideal opportunity to enhance the training of many individuals in the country since the basic infrastructure and capacity was already in existence.

The grant entered into by ABSP and AGERI focused toward the ultimate goal of production of a variety of selected crops (initially specifically potatoes, maize, cucurbits and tomatoes) that showed resistance to major economic pests and diseases in Egypt. Since 1993, ABSP's collaboration with AGERI has continued to evolve in applied research and has also examined the important policy areas of biosafety and Intellectual Property Rights. In addition to its product-oriented focus, the collaboration has explored appropriate technology transfer, human resource development and links between the public and private sectors in both countries. The project has thus encompassed research, policy, networking and management aspects of biotechnology.

A Final Report on the first phase of the project was submitted in 1995. This report therefore outlines the activities and achievements of the* Commercialization and Utilization of Biotechnology (CUB) Project *[263-0240-G-00-6014-00] for the period from 1995 to 2002.

Project Goals

The overall goal of the ABSP/AGERI collaboration, under the Commercialization and Utilization of Biotechnology (CUB) project, was ***to improve the capacity and policy environment for the use, management, and commercialization of agricultural biotechnology in Egypt.***

This goal was structured to fit within USAID Cairo's overall goal of broad-based sustainable development with increased employment and improved quality of life, and the first strategic objective of USAID's Cairo Mission—*accelerated private sector-led, export oriented economic growth.*

Specific project goals were i) to mutually enhance US and Egyptian institutional capacity for the use and management of biotechnology; ii) to develop environmentally compatible, improved germplasm; iii) to build linkages with the private sector; and iv) to test and transfer technologies to the field. These goals were to be achieved by addressing two primary objectives:

1. **Establishment of a policy framework in Egypt that promotes the use, management and commercialization of biotechnology by AGERI and other agricultural institutions and enterprises.**

   Purposes:
   - *To foster and increase the capacity of the Egyptian agricultural sector in biotechnology through the establishment of linkages with advanced laboratories in the US (both public and private).*
   - *To facilitate field-testing, product development and commercialization of usable products for the mutual benefits of Egypt and the US.*
   - *To enable Egyptian scientists to develop sound knowledge of intellectual property rights, and apply it effectively in product development.*

2. **Improvement of marketed crops through strategic research partnership between AGERI and US institutions (both public and private sector).**

   Purpose
   - *To develop and test commercially important plant materials.*

Mid-Project Evaluation

An interim evaluation of the CUB project was carried out for USAID in June 1998 by Dr. Donald Plucknett (*Agricultural Research and Development International*). As part of this evaluation Dr. Plucknett visited MSU, Pioneer Hi-Bred, Cornell University and AGERI, and attended the 1998 Project Workshop held in Cairo. Dr. Plucknett's report made several recommendations that were taken into consideration in developing the final phase of the project, which began in 1998. The main recommendations in the report concerned moving forward from the research and development stage towards moving improved materials into farmer's fields.

In order to better achieve this goal, the report recommended that increased effort be placed on strengthening the institutional capacity of AGERI to handle the products of agricultural biotechnology. Specifically this included refocusing the research projects towards those more likely to produce a commercial product in the relatively short-term, the establishment of a Technology Transfer Office at AGERI for management of IP issues, and by providing assistance to AGERI in developing strategic plans for the commercialization of products. More emphasis was to be placed on developing linkages with the private sector, and in assisting AGERI in developing long term plans for self-sustainability.

Expected Outputs of project

Products:

- Genetically engineered insect and virus resistant food and horticultural crops (maize, potato, cucurbits, tomato), greenhouse and field-tested.
- A strong IPR base and application of appropriate legal frameworks for commercial development.
- Egyptian authorship on technical, peer-reviewed publications.

Institutional:

- At least 15 Egyptian scientists cooperating in advanced agricultural biotechnology programs at US universities and private sector companies.
- Industry-based management study tour for Egyptian senior scientists/managers.
- Increased Egyptian institutional capacity in intellectual property and biosafety.

Project Indicators

The success of the project was to be measured by the following indicators:

- *At least two genetically engineered cultivars with enhanced agronomic qualities and/or pest and disease resistance, developed by CUB or external sources (e.g. multinational seed companies) are field-tested and undergoing registration (the first step to commercialization) in Egypt.*
- *Science-based policies for IPR, biosafety and/or novel foods drafted and adopted at national and/or institutional levels.*
- *Applications for field-testing and/or protection of intellectual property rights for improved and/or genetically engineered varieties handled effectively by Egyptian regulatory bodies.*
- *Private and public sector partnerships identified and agreements signed.*
- *AGERI capable of accessing external technology and marketing improved technologies*
- *Multinational companies applied for field testing in Egypt; review system in place and functioning*

Summary of Achievements

Under the CUB/AGERI collaborative project in agricultural biotechnology, Egypt has made excellent progress both in policy (intellectual property rights and biosafety) and research areas. In terms of policy developments, Egypt has adjusted its national intellectual property rights (IPR) policies to include the patenting of food and plant products. A Plant Variety Protection law (PVP) is pending the approval of the Egyptian parliament. Egypt has approved biosafety guidelines and developed an effective framework with which to enforce these guidelines. The scientists at AGERI have developed awareness of the importance of IPR, biosafety and technology transfer policies and their implementation both at the national and institutional level. AGERI scientists have been successful in the development of transgenic plants, which have been tested in the greenhouse and fields for research purposes. These impressive achievements now set an excellent stage for the future commercialization of the products of agricultural biotechnology in Egypt.

Through its support to AGERI, MSU and its partners have enabled the development of important new agricultural technologies and continued policy reform to improve the transfer and management of these technologies by AGERI and the private sector. Significant progress has therefore been made toward the original ABSP/AGERI goal of mutually enhancing US and Egyptian institutional capacity for the application and management of biotechnology.

Some of the major achievements of the CUB project in Egypt are summarized below.

Major achievements:

- ***Transgenic potatoes developed and successfully field-tested in Egypt.***
- ***Transgenic and traditionally bred cucurbits developed and successfully field-tested in Egypt.***
- ***Development and adoption of biosafety regulations in Egypt.***
- ***National Biosafety Committee established, biosafety review system in place and functioning.***
- ***Egyptian Plant Variety Protection legislation drafted.***
- ***Multinational companies applied for field-testing of transgenic products in Egypt (e.g. Pioneer Hi-Bred, Seminis).***
- ***Technologies developed at AGERI have been patented and licensed.***
- ***Research collaborations developed successfully with the private sector (Pioneer Hi-Bred).***

Objective 1.

Establishment of a policy framework in Egypt that promotes the use, management and commercialization of biotechnology by AGERI and other agricultural institutions and enterprises

The first objective of the CUB project ***- Establishment of a policy framework in Egypt that promotes the use, management and commercialization of biotechnology by AGERI and other agricultural institutions and enterprises*** – was addressed largely via policy assistance and institution building within AGERI.

The three main thrusts to this policy framework were:

- **Biosafety;**
- **Intellectual Property Rights (IPR); and**
- **Commercialization/institution building.**

BIOSAFETY

Biosafety is now the number one issue of concern of developing countries in the development and application of agricultural biotechnology. This high priority is most likely a result of the International Biosafety Protocol (the Cartagena Protocol on Biosafety) and issues surrounding public acceptance of the technology. The ABSP placed a high priority on the establishment of science-based regulatory structures for the promotion of technology access for Egypt. Long before the international awareness of biosafety, the ABSP has been working with our developing country collaborators to provide technical assistance and training to promote national decision-making.

Within the ABSP itself, before any genetic transformation technology or materials may be transferred, the recipient country must have in place a regulatory approval mechanism to insure the safe transfer, handling and permitting of transgenic materials. Linking the development of an important agricultural product to biosafety policy assistance has facilitated important policy changes within our partner countries. Through a combination of workshops, one-on-one consultations, and longer internships, the ABSP has promoted the development of sound biosafety systems at both the national and institutional levels in Egypt. The success of this assistance as described below, linked to the ABSP research program, is one of the major achievements of the ABSP.

The ABSP/AGERI collaboration placed a very high priority on biosafety, both in terms of awareness and implementation. Supported by a series of internships, consultations and workshops, biosafety in Egypt has been strengthened through the development of biosafety guidelines, training of scientists and in the principles and practice of biosafety review, and construction of a biocontainment greenhouse facility.

Egypt is among the developing countries most advanced in the adoption and use of agricultural biotechnology. AGERI's mandate – to develop transgenic products tailored for local conditions and consumer preferences – clearly indicated the need for the development of a regulatory system. Additionally, multinational companies have been seeking permission to import their GMO crops for testing in Egypt since 1995.

Overview of Activities

Under the first phase of the CUB project (1991-1995) several initiatives in biosafety capacity development were undertaken with AGERI.

This began in May 1993 with the ***Biosafety Internship Program***, an eight week program in the US with the goal of assisting collaborating countries in the development of biosafety guidelines that would allow them to exchange and test biotechnology products. Seven scientists from Egypt, as well as those from other ABSP collaborating countries participated in the program at Michigan State University, followed by a visit to Washington DC where they had the opportunity to interact with federal personnel at USDA/APHIS, FDA and EPA responsible for various aspects of biosafety.

Following the internship program a ***Biosafety workshop*** was held at AGERI in January 1994 in order to create a greater awareness and strengthen the biosafety regulatory framework in Egypt and the Middle East. The workshop involved international experts in biosafety, and scientists and regulatory personnel from Egypt and selected countries in Africa and addressed policy, risk assessment and field-testing issues surrounding the management and safe handling of transgenic plants. Dr Patricia Traynor, ABSP Biosafety Consultant, was brought in by the CUB project to review and provide comments on the draft biosafety guidelines developed by the Egyptians.

In 1995 ABSP provided leadership in the development of a subcontract to the AGERI/ABSP collaboration with the University of Arizona for the construction of a ***BLP-2 containment greenhouse facility*** at AGERI. Certification of the containment facility was authorized by the Chief of Microorganisms Branch at the USDA/APHIS/BBEP. As well as being the first of its type in Africa (outside South Africa), the AGERI biocontainment facility was a model for the facility subsequently constructed in Indonesia through World Bank funding.

Since 1995, CUB project biosafety activities have focused primarily on individual training of those at AGERI responsible for the biosafety process, and also on obtaining the necessary biosafety clearances for transfer and field-testing of transgenic crops developed under the CUB project, specifically the Bt potato tuber moth-resistant potatoes developed at MSU.

Biosafety Internship, 1995: The AGERI biosafety Officer, Mr. Ahmed Wally, attended a two-week internship program in August 1995, which focused on field-testing genetically engineered plants. Mr. Wally became familiar with the procedures for risk assessment, preparing permit applications, and conducting field tests.

Biosafety Internship, 1996: In August 1996, Dr. Taymour Nasr El-Din and Mr. Khaled Essam from AGERI took part in a two-week internship program on the biosafety review process. Particular emphasis was placed on assessing the potential for environmental consequences, and risk management issues.

Food Safety Training, 1999-2002: Michigan State University (MSU) conducts a one-week short course with a focus on food safety policy development, risk analysis, and program implementation. Several ABSP-sponsored participants from Egypt have attended this course since its inception in 1999.

Food Safety Consultancy, 2001: ABSP assisted in the recruitment of Dr. Hector Quemada, Crop Technology Inc., as a special consultant to assist the government of Egypt in the development of food safety guidelines and regulations for foods derived from GMOs. ABSP also assisted in the development of the scope of work for this assessment. USAID/Cairo supported this activity through the DAI/APRP policy project.

Achievements/Outputs

INDICATOR: SCIENCE-BASED POLICIES FOR IPR, BIOSAFETY AND/OR NOVEL FOODS DRAFTED AND ADOPTED AT NATIONAL AND/OR INSTITUTIONAL LEVELS.

- *A National Biosafety System was instituted by the Ministry of Agriculture and Land Reclamation in two decrees issued in 1995. Ministerial Decree No. 85 (January 25, 1995) established a National Biosafety Committee (NBC); Ministerial Decree No. 136 (February 7, 1995) adopted biosafety regulations and guidelines for Egypt. The system involves several ministries, organizations and/or government agencies involved with the importation, exportation and local production of natural products. The guidelines describe the modalities of use, handling, transfer, and testing of transgenic organisms. They address laboratory practices, greenhouse containment, and small-scale field-testing.*
- *Procedures for commercial release of transgenic plants in Egypt established in 1998 by Ministerial Decree No. 1648.*
- *Development of Egyptian food safety laws/regulations currently underway.*
- *AGERI Biosafety Officer trained and drafted biosafety guidelines for laboratory, greenhouse and field experiments.*
- *Biocontainment greenhouse facility constructed at AGERI.*

INDICATOR: APPLICATIONS FOR FIELD-TESTING AND/OR PROTECTION OF INTELLECTUAL PROPERTY RIGHTS FOR IMPROVED AND/OR GENETICALLY ENGINEERED VARIETIES HANDLED EFFECTIVELY BY EGYPTIAN REGULATORY BODIES.

- *Field-testing of GMOs from both public and private sector conducted in Egypt: including*
  - *i. Insect-resistant potatoes (MSU and AGERI)*
  - *ii. Virus resistant squash (AGERI);*
  - *iii. Virus resistant tomatoes (AGERI); and*
  - *iv. Bt resistant maize (Fine Seeds/Novartis, Pioneer Hi-Bred).*

INDICATOR: MULTINATIONAL COMPANIES APPLIED FOR FIELD-TESTING IN EGYPT; REVIEW SYSTEM IN PLACE AND FUNCTIONING.

- *Field-testing of GMOs from private sector conducted in Egypt: including*
  - *i. Bt resistant maize (Fine Seeds/Novartis),*
  - *ii. Bt maize (Pioneer Hi-Bred), and*
  - *iii. Bt Maize (Verneuil Semences).*

IPR & TECHNOLOGY TRANSFER

From its inception, the ABSP has supported capacity building and policy assistance in Intellectual Property Rights (IPR) and technology transfer. In the design of the ABSP, USAID realized that new technological breakthroughs, private investment, and governmental protection of intellectual property were spurring agricultural biotechnology in developed countries. This has led to a changed institutional structure of agricultural research with a greater collaboration between public institutions and private biotechnology companies.

Historically, national programs in developing countries have relied on public sector institutions in developed countries for advances in basic research, which are then adapted for application. The traditional route of donor and developing country access to biotechnology through US public institutions, once a timely and responsive way of conducting agricultural research geared towards developing countries' needs, has been altered by increasing domestic trends towards privatisation of research. On the international level, biotechnology industries are being established in many developed countries and are supported by increased protection of intellectual property (i.e. TRIPS Agreement). Consequently, the ABSP was designed to assist developing countries in accessing proprietary germplasm and technologies relevant to crops of commercial significance.

The ABSP has taken a number of approaches to intellectual property issues, at the national and institutional level in Egypt. The ABSP philosophy has been to provide information and training, and facilitate countries and institutions in the development of their own strategies, policies and procedures to access proprietary technology. The ABSP has held one-on-one consultations, workshops, and internships to provide assistance to Egypt. Additionally, ABSP has sponsored participation to international meetings and access to news and information on IPR via the World Wide Web. Capacity building in IPR is one of the areas in which the ABSP has achieved unique success, and can serve as an effective model for other programs in agricultural biotechnology and development.

The overall goals in Technology Transfer in this phase of the CUB project were:

- The Establishment of a pilot technology transfer office at AGERI

  The development of a technology transfer office (TTO) would give AGERI the capacity to handle IPR concerns internally. Activities to be handled within this office include the management of new inventions, the protection of these inventions, the identification of partners for development and the marketing of inventions. This office would serve both to market technologies developed within AGERI, and also to seek out new partnerships and technologies that will assist AGERI in its scientific and development mission. This effort was viewed as a pilot operation to demonstrate to the ARC and others the merit of this type of model.

- Establishment of commercial linkages

  ABSP and AGERI have continued throughout the project to build upon established private sector linkages. More such linkages should be encouraged in addition to the already established contractual linkage with Pioneer Hi-Bred, and an informal relationship with Seminis Vegetable Seeds. ABSP/AGERI will continue to work with the Egyptian Seed sector to promote linkages.

Overview of Activities

In the first phase of the CUB project, Technology Transfer/IPR activities largely involved training in the form of internships and workshops. The first of these was the ***Intellectual Property/Patent Internship Program***, held at Stanford University, in 1993. Designed and implemented by Professor John Barton of Stanford Law School, the program was attended by interns from Egypt, Kenya and Indonesia. The goal of the program was to provide hands-on experience to legal and scientific personnel from developing countries in various issues related to intellectual property rights. This was followed in January 1994 by a ***Workshop On Intellectual Property Rights, Patents & Licensing***, held in Cairo, Egypt. Again designed by Prof. John Barton, the workshop was attended by 100 participants from various public and private sector institutions, the goal of which was to create a greater awareness among the Egyptian scientific community in the various issues relating to intellectual property in agricultural biotechnology. The workshop involved scientists, legal professionals and government officials from Egypt.

In the second phase of the CUB project, training and capacity building in IPR and Technology

Transfer continued, but a major priority has been assisting AGERI in setting up their Technology Transfer Office, and in managing the biosafety and intellectual property issues relating to the transgenic products of CUB research projects, specifically the Bt potato tuber moth resistant potatoes developed at MSU.

Technology Transfer activities in this phase of the CUB project are summarized below.

Support of Egyptian Participants to Attend MSU Intellectual Property Rights and Technology Transfer Internship Program 1996-2002: The MSU Institute of International Agriculture, in cooperation with the Office of Intellectual Property and ABSP operates an annual two-week internship program in Intellectual Property Rights and Technology Transfer. The program is aimed at providing hands-on experience to international participants in various aspects of intellectual property rights and technology transfer including patents, plant variety protection/certification, trademarks, copyrights and trade secrets. The Internship Program fosters South-South collaboration by providing a forum for the participants to present the status of IP management within their own countries, followed by a facilitated discussion. Nineteen Egyptian participants, many of whom have been involved directly with the CUB project have attended this internship since 1996.

Intellectual Property Rights Seminar on the Legal Framework for Technology Transfer, Egypt 1995: Under the auspices of the American Embassy in Cairo, the ABSP/AGERI project assisted in the organization of a two-day seminar on the legal framework for technology transfer. The seminar focused on intellectual property rights and technology transfer issues within the context of recent changes in GATT. Over 100 representatives from government and private sector institutions in agriculture and the pharmaceutical industry attended the workshop.

Linkages with the Association of University Technology Managers (AUTM), 1995-2002: In order to build intellectual property management and technology transfer capacity in collaborating countries, the ABSP project has since 1995 developed close links with the Association of University Technology Managers (AUTM) in the US. The AUTM is a professional association of technology transfer managers from academia, government institutions and industry. The ABSP Technology Transfer Coordinator has attended the annual meeting of AUTM since 1995, and ABSP has sponsored participants from several developing countries, including four from Egypt to attend the annual or regional meetings of AUTM in the US. In 2002, ABSP sponsored their first international booth at the AUTM Technology Transfer Fair. At the meeting, AGERI displayed information about its TT offices and the technologies that are available for licensing.

Transfer and Field Testing of Transgenic Potatoes in Egypt, 1998-2003: Potato tuber moth (PTM) resistant Bt transgenic potato lines developed under the ABSP/AGERI project have been transferred to Egypt under appropriate material transfer agreement for field testing and evaluation in each year of the project since 1998. The ABSP project complied with all the biosafety and legal requirements for the transfer of these lines to Egypt for research purposes. Dr Karim Maredia, ABSP Technology Transfer Coordinator, traveled to Egypt in December 1997 to finalize the plans for the first field test, and to facilitate the signing of the Material Transfer Agreement. The permit application for the field-testing of the potatoes was approved by the Egyptian National Biosafety Committee (NBC) in December 1997. Relevant documentation for approval was also submitted to USAID Washington and USAID Cairo. The potatoes have subsequently been field-tested annually both at the AGERI field site in Giza and at the CIP field station in Kafr-El-Zayat.

ABSP/AGERI Project Workshop, Cairo, May 1998: The ABSP/CUB collaborative project hosted a project evaluation workshop at AGERI in Cairo from May 18-20, 1998. This very successful workshop had several goals. It provided a means for Egyptian and US scientists to present their

collaborative research work; it provided a forum for public and private researchers to share ideas and concerns, including concerns regarding Intellectual Property Rights, Biosafety, marketing and technology adoption; and it provided an opportunity for discussion of research needs and future collaborative plans. Private sector participants included individuals from Pioneer Hi-Bred, Aventis, Seminis, and Hy-Tec.

Support to Technology Transfer Office, AGERI May 1999: Dr. Magdy Madkour and Dr. Mohamed Eid from Egypt visited MSU in May 1999. They interacted with the MSU Office of Intellectual Property Rights (OIP). Based on the information provided during their visit, Dr. Eid has drafted an Intellectual Property Rights (IPR) policy for the Agricultural Genetic Engineering Research Institute (AGERI). A copy of this policy has been sent to MSU for review and comments, and forwarded to USAID. The IP policy will serve as the basis for the technology transfer program at AGERI and eventually all the agricultural research centers in the Ministry of Agriculture.

CUB Egypt Symposium, May 2000: 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.

Transfer of Virus Resistant Cucurbits Germplasm from the US to Egypt, March 2000: In March 2000, under an appropriate MTA, Dr. Molly Jahn, Cornell University transferred 29 lines of squash, melon, and cucumber to Egypt. These materials have been successfully evaluated in the field against virus diseases in Egypt.

Intellectual Property 'Audit' for Bt Potatoes, 2001: During 2001, Dr. Fred Erbisch, completed an Intellectual Property 'audit' of MSU's potato tuber moth resistant B.t. potatoes. This is another step in the process of obtaining legal clearances to commercialize MSU's transgenic potatoes in Egypt and South Africa, as well as any other developing countries.

IPR/Legal clearances for potatoes, 1998-2002: ABSP has continued to work closely, with Zeneca and Syngenta in IPR negotiations for the Cry5 Bt gene, which is utilized in the MSU potato tuber moth resistant potatoes. This is an important step towards developing a road map for regulatory approval and IP clearance for commercialization of these B.t. potatoes in Egypt and South Africa.

Support to ARC Technology Transfer Office, ARC 2001: Dr. Fred Erbisch, MSU adjunct professor and ABSP consultant, spent 3 weeks in Egypt during the summer assisting the Agricultural Research Center (ARC) in developing basic materials and policy for its planned technology transfer office.

IPR and Plant Variety Protection (PVP) Training at MSU, September 2001: As a result of the consultation in Egypt for the ARC (Ministry of Agriculture), Drs. Erbisch and Karim Maredia, with ABSP staff support, developed and participated in two additional short-term training programs at

Michigan State University in September 2001. One program was on intellectual property management and was presented to 9 representatives from the Egyptian ARC. The ARC will now establish a technology transfer office in Egypt that the September workshop participants will operate. The second training program dealt with plant variety protection (PVP) and was attended by 8 senior representatives from the ARC. Participants of this workshop will be staffing the Egyptian PVP Office.

Achievements/Outputs

INDICATOR: SCIENCE-BASED POLICIES FOR IPR, BIOSAFETY AND/OR NOVEL FOODS DRAFTED AND ADOPTED AT NATIONAL AND/OR INSTITUTIONAL LEVELS.

- *ESTABLISHMENT OF THE TECHNOLOGY TRANSFER OFFICE AT AGRICULTURAL GENETIC ENGINEERING RESEARCH INSTITUTE (AGERI) 2001: The Office of Technology Transfer and Intellectual Property (OTTIP) at AGERI was established. Internal IP policy was developed and approved. A model Material Transfer Agreement (MTA), a License Agreement and a Confidential Disclosure Agreement were developed, based on MSU Office of Intellectual Property forms, in both English and Arabic and a comprehensive awareness program for AGERI staff was implemented. This effort makes AGERI one of only few developing country institutions to adopt policies and procedures for management of intellectual property rights.*

- *ADOPTION OF TECHNOLOGY TRANSFER POLICY WITHIN THE MINISTRY OF AGRICULTURE (ARC) 2001: In addition to developing IP policy at AGERI, the OTTIP has been instrumental in developing an IP policy for the Agricultural Research Center (equivalent to an Agricultural Research Service/USDA policy). The ARC has more than 10 research institutions covering a wide range of agricultural research, including mechanization, pesticide research and horticulture. This ARC policy makes Egypt one of the only developing countries to have developed a government strategy on the management of intellectual property rights in agriculture.*

- *ESTABLISHMENT OF AN INTELLECTUAL PROPERTY RIGHTS (IPR) CENTER AT MENOUFIA UNIVERSITY, 1999: Through IPR training provided in Cairo in April 1999 for Professor Ibrahim Siddik, Vice President for Community Services, the Menoufia University in Egypt established a new IPR Center in the Faculty of Law. This new Center provides IPR related legal services to the university community. Menoufia University has 17 colleges/institutes with approximately 2,000 faculty members and 60,000 students. The establishment of intellectual property rights services within the university community in Egypt is an important extension of ABSP's efforts to establish IP management expertise and assistance to scientists in the developing world.*

INDICATOR: PRIVATE AND PUBLIC SECTOR PARTNERSHIPS IDENTIFIED AND AGREEMENTS SIGNED.

- *The most significant research and training partnership in the CUB program was that developed with Pioneer Hi-Bred.*
    - *i. Several researchers from AGERI received short and longer-term training at Pioneer Hi-Bread, Iowa.*
    - *ii. AGERI and Pioneer signed research agreements concerning the transfer of promoters, strains of Bacillus thuringiensis and other research tools both to Pioneer, and to AGERI.*

iii. Relationship with Pioneer led indirectly to the applications by other private sector companies to field test transgenic crops in Egypt.

INDICATOR: AGERI CAPABLE OF ACCESSING EXTERNAL TECHNOLOGY AND MARKETING IMPROVED TECHNOLOGIES

- *Through appropriate Material Transfer Agreements, AGERI has obtained transgenic plant materials (potatoes, tomatoes and cucurbits) for research purposes and field-testing from MSU, and non-transgenic improved materials from Cornell University.*
- *AGERI and Pioneer signed research agreements concerning the transfer of promoters, Bt genes etc. to AGERI.*
- *In 2002 AGERI participated in the annual meeting of the Association of University Technology Transfer Managers (AUTM), AGERI displayed information about its Technology Transfer offices and the technologies available for licensing.*
- *The reports from the Haas Business School studies identified various marketing opportunities for AGERI, which are now being further investigated.*

COMMERCIALIZATION & INSTITUTIONAL DEVELOPMENT

One of the overall goals of the Commercialization/Technology Transfer/Institution Building portion of the initial proposal was to assist AGERI in long-term strategic planning and in developing a vision and plan for sustainable growth. This has been accomplished by a number of initiatives in this phase of the project, including:

- Development of management skills.

  CUB supported the development of management skills for personnel at AGERI and provide for management/private sector consultants to assist AGERI in transitioning from a public-sector research institute to a more commercially operating unit.

- Development of privatization feasibility and strategic plans for the future of AGERI.

  CUB provided funds for assistance to AGERI in developing privatization feasibility studies and a long-term strategic plan for the sustainability of AGERI, post-USAID support. This activity was conducted by the University of California's Business School, an internationally recognized leader in business consulting. USAID/Cairo and the ARC, in order to assist AGERI in developing a sustainable funding base and continued future, expressly requested these studies.

In its discussions on the future of AGERI, USAID/Cairo and the Agriculture Research Center (ARC) suggested that the Institute consider privatization. ABSP collaborated with University of California – Berkeley's Haas Business School to assist AGERI in analyzing options for privatization and developing a strategic plan in order to become institutionally self-sufficient. ABSP assisted AGERI in three ways:

- Assisting AGERI staff in developing management skills.
- Engaging a biotechnology business consultant to assist AGERI in implementing its strategic plan.
- Coordination with the University of California – Berkeley's Haas Business School to develop a privatization feasibility and strategic plan.

Overview of Activities

Three-year project agreement between Haas School of Business and ABSP, 1999-2001: The three-year project assessments took place from 1999-2001. Project teams from the Haas School of Business, International Business Development Program (IBD), University of California, Berkeley, conducted an analysis in each of the 3-years on the external and internal environment in which AGERI operates. The IBD, in its eleventh year, is a renowned MBA consulting program where small teams of graduate students work with clients on projects in overseas locations.

Commercialization Prospects for AGERI, 1999: The Haas team's research focused on the organization's financial costs and revenue streams. The assessment was two-fold. In the first part of the assessment, 28 interviews with a broad spectrum of the biotechnology industry and the agriculture sector, were conducted to gain insight into different aspects of AGERI's operational environment, to test and evaluate the knowledge about genetic research in agriculture, to assess the willingness to work with GMOs and to explore AGERI's future business opportunities. The second part of the research entailed an analysis of possible demand for AGERI projects and estimates of the resulting revenue streams. Following completion of the assessment, the team made six recommendations to AGERI. They included: 1) secure continued funding to meet the organization's $1.5 million operating costs; 2) allocate resources according to relative profitability; 3) evaluate the business strategy and clarify the organizational mission; 4) provide organizational structure to support the AGERI mission; 5) develop a strategic marketing plan; and 6) consider long-term tasks and prospects.

Strategic Marketing Plan for AGERI, 2000: A second Haas team, under the guidance of Drs. Catherine Ives, Director, ABSP and Magdy Madkour, Director, AGERI, developed a strategic marketing plan for AGERI The team looked at various players in the agricultural biotechnology sector and at issues relating to genetic engineering of food crops. Thirty-five interviews were conducted at AGERI with management and principal investigators, with international business, a domestic seed company and international and Egypt government related agencies. They also looked at published and unpublished statistical data from a range of sources. Some over-arching recommendations for AGERI were: 1) advance a market-driven philosophy; 2) expand management capability; 3) build strategic partnerships, especially through the local seed industry; 4) expand external communication efforts; 5) select the highest potential crops and traits; 6) evaluate projects based on a market-based metric; 7) promote international standards of intellectual property protection (IPR) and biosafety regulation; 9) encourage a public awareness campaign, and 9) grow the underutilized GESU (the Genetic Engineering Services Unit at AGERI) as an additional source of revenue.

Preparing AGERI for Continued Success in the Evolving Biotechnology Industry, 2001: The third Haas team investigated prospects related to AGERI's internal organization. The assessment was again in two phases that included: 1) preliminary research in the USA involving an in-depth review of the previous two reports, industry and country analyses, discussions of the project with various US faculty, industry professionals and Egyptian-Americans; and 2) extensive interviewing during a 3-week period in Egypt with management, scientific staff and other key personnel and industry representatives. Fifty-one interviews with current AGERI employees were conducted with the goal of obtaining a broad sample of the staff. The research results found that although AGERI has successfully developed a unique culture and a solid foundation of research and that the scientific staff is passionate about their work, there are many areas in need of remediation, especially in the area of communication. The final recommendations include: 1) finish developing the mission, vision and values statements; 2) establish a broader organizational framework within AGERI; 3) use a standard project proposal framework with scientists; 4) identify critical skills that

all AGERI employees should develop, a clear path for advancement and a performance appraisal system; 5) establish a scientific advisory council and annual research symposium; 6) develop a market-focus to guide project selection of research and 7) pursue an endowment funded by USAID.

SPECIALIZED TRAINING

Training at MSU, 2002: Since AGERI's inception, the facility put in place fully equipped laboratories and made adjustments to the structure of the labs and ancillary facilities as necessary to keep up with the current scientific research. The functional genomic laboratory is one of these specialized laboratories. Dr. Dina El-Khishin, Researcher and Head of the genomic unit and Dr. Walid Maaty, Researcher and proteomics specialist, spent three months working in collaboration with Dr. Joseph Leykam, Manager, Genomic Technology Support Facility in the MSU Plant Biology Laboratory. Dr. El-Khishin and Dr. Maaty also provided leadership on the procurement of state-of—the-art equipment to support the expanded role of genomics, proteomics and bioinformatics at AGERI. The new equipment has been received at AGERI and is currently being tested and installed. Dr. El-Khishin remarked during her visit to MSU that it is their wish to be a resource for genomics and proteomics analysis to the Middle East and Northern Africa.

Objective 2.
Improvement of marketed crops through strategic research partnership between AGERI and US institutions (both public and private sector).

One of the most important components required to improve the agricultural sector in Egypt is the continued investment in technology improvements. Given Egypt's limited land and water, and its increasing population, increases in agricultural production and yield improvements will only come with advances in technology. Biotechnology is one important set of tools that can be used to increase production and improve quality, while protecting the country's natural resource base.

Research collaborations have been an important component of the CUB, as it is critical to continuously develop and adapt new technologies and to assist Egyptian scientists to develop and use new techniques and apply them to Egyptian crops and agricultural conditions. These research collaborations have continued to be successful throughout the life of the project, and have addressed the second major objective***—Improvement of marketed crops through strategic research partnership between AGERI and US institutions (both public and private sector)***.

The applied research partnerships initially supported by the CUB project were:

- Development of virus resistant cucurbit crops using a combination of molecular genetic and conventional breeding approaches.
  Michigan State University; Cornell University, AGERI
  Cucurbits (melon, watermelon, squash and cucumber) are an important export crop for Egypt and a focus crop of the USAID/Cairo associated project, Agricultural Technology Utilization and Export (ATUT). They are currently devastated by virus infection and this project, through a combination of research tools including biotechnology and traditional breeding, seeks to develop varieties that are resistant to viruses, adapted to Egyptian conditions, and suitable for export to Europe.
- Development of insect resistance in commercial Egyptian maize.
  Pioneer Hi-Bred; AGERI
  This collaboration involved a formal relationship with Pioneer Hi-Bred, the world's largest maize seed company. The project aims to develop maize that is resistant to an important insect in Egypt using biotechnology. Direct collaborations with Pioneer, which has a seed distribution business in Egypt, will facilitate commercialization of the improved maize lines.
- Potato transformation for development of potato tuber moth resistance.
  Michigan State University; AGERI; CIP-Egypt.
  The potato tuber moth is an important insect pest in Egypt, and development of resistant varieties will greatly reduce yield losses, increasing farmer incomes, and assist in the development of a potato seed industry. Potential Egyptian private sector collaborators (both growers and tissue culture companies) have been approached to assist in further evaluation of these materials.
- Production of tomato yellow leaf curl virus (TYLCV) resistant tomato
  ILTAB, Scripps Research Institute; AGERI
  The aim of this project was to develop strategies and reagents for the diagnosis of such viruses for use in Egypt and other relevant regions of the world, and also to develop plants by genetic transformation that are resistant to tomato yellow leaf curl virus (TYLCV) a major disease of tomatoes in Egypt.

- Molecular characterization of insect midgut toxin receptors for circumventing resistance to toxins of Bacillus thuringiensis.
  University of Wyoming/University of Texas-Dallas, AGERI
  This collaboration continued the successful partnership between AGERI and Dr. Lee Bulla (who has recently moved to the University of Texas-Dallas). The development of new insect toxins is crucial to the long-term sustainability of agricultural biotechnology. By understanding the underlying molecular mechanisms of resistance, new genes can be identified or manufactured that are toxic to insects. This collaboration has already resulted in the successful commercialization of a biopesticide in Egypt.

  Improving quality and marketability of tomato in Egypt.
  AGERI
  This project focuses on the continued development of transgenic tomatoes with putative resistance to geminiviruses, a devastating virus of tomatoes and other crops within the Middle East. AGERI is now equipped to take the lead on this work, having received significant assistance from Scripps Research Institute in the previous grant period. AGERI will explore links with the private sector (Seminis Seed Company) for testing and screening resistant tomato plants.

- Engineering crop plants with resistance to drought and salinity
  Ohio State University, AGERI.
  This collaboration involves the development of drought resistant tomato. Obviously, Egypt will need to develop crops that are resistant to drought and tolerant to salt. Although a difficult technical problem, the science has progressed enough in the last few years to make this a reasonable approach. *This project was added to the research portfolio in 1998.*

Achievements/Outputs

INDICATOR: *AT LEAST TWO GENETICALLY ENGINEERED CULTIVARS WITH ENHANCED AGRONOMIC QUALITIES AND/OR PEST AND DISEASE RESISTANCE, DEVELOPED BY CUB OR EXTERNAL SOURCES (E.G. MULTINATIONAL SEED COMPANIES) FIELD TESTED AND UNDERGOING REGISTRATION IN EGYPT.*

- ***Potatoes***: Several lines have been field-tested in successive years and shown to have resistance to potato tuber moth in the field, and subsequently in storage, including:
  - *Spunta G2 and Spunta G3, from a locally grown Egyptian variety transformed with a Cry5 vector.*
  - *Lemhi Russet transformed with Cry1A(c) and Cry5*
  - *Atlantic transformed with Cry5*
- ***Cucurbits**:*
  - **Transgenic Melon cultivars** e.g. Hale's Best Jumbo, Topmark, Ananas El Dokki, Shahd El Dokki with resistance to zucchini yellow mosaic virus using the ZYMV coat protein have been field-tested in Egypt. (AGERI)
  - **Transgenic Eskandarani squash**, transformed with the coat protein gene of ZYMV greenhouse have been field-tested in Egypt. (AGERI)
  - **Conventionally-bred lines of *Cucurbita pepo*** with resistance to one or more viruses have been field-tested in Egypt. (Cornell University).

- **Conventionally-bred Cucumber** -- Breeding lines created in Beit and Asian types with various combinations of conventional resistance to the following diseases and pests: four viruses, three leafspots, scab, reduced attractiveness to cucumber beetles, powdery and downy mildew, have been field-tested in Egypt, as well as in the Philippines, Indonesia, S. Africa, and Brazil. (Cornell University)

- ***Transgenic Tomato:*** Lines engineered for resistance to geminiviruses using the coat protein, the pre-coat protein, the replicase gene or the AV4 gene of Egyptian geminiviruses have been greenhouse and field-tested in Egypt.

- ***Transgenic Maize****:* Field-testing of GMOs from the private sector have been conducted in Egypt: including
  - Bt resistant maize (Fine Seeds/Novartis),
  - Bt maize (Pioneer Hi_Bred), and
  - Bt Maize (Verneuil Semences).

Short (one to two page) summaries of each of the research projects are given below, followed by the full final technical reports.

RESEARCH SUMMARIES

MAIZE TRANSFORMATION FOR DEVELOPMENT OF STEM BORER RESISTANCE IN TROPICAL MAIZE.

Pioneer Hi-Bred, USA
Agricultural and Genetic Engineering Research Institute (AGERI), Egypt

1996-2001

Project Goals

Maize stem borers (*Sesamia cretica, Ostrinia nubilalis, Chilo agamemnon)* are serious insect pests in much of the maize growing area of Egypt and the Middle East and are responsible for significant loss of yield. Application of chemical pesticides has been the only contact measure taken against these insects. The overall goal of the project is to introduce into Egyptian commercial corn varieties Bt gene(s) that are known to code for proteins that are lethal to these lepidopteran species. The specific objectives of the project are as follows:

i. Transfer technologies from U.S. counterpart to establish a system(s) for regeneration and transformation of Egyptian maize lines.

ii. Production of genetically engineered maize elite resistant to stem borers specifically *Sesamia cretica* (pink corn borer), via transformation with an insect resistance endotoxin Bt gene.

iii. Develop laboratory rearing for the lepidopteran pink borer, *Sesamia cretica*.

iv. Establish methods for laboratory bioassays and field-testing.

Project Impacts

This research collaboration between Pioneer Hi-Bred and AGERI has progressed significantly. Important accomplishments include the development of regeneration and transformation systems for elite Egyptian maize lines, coupled with training of four Egyptians in molecular biology, cell culture and transformation and exposure to intellectual property and regulatory issues. An effective system was developed for the regeneration and transformation of Egyptian maize lines, a laboratory rearing facility was successfully developed, and methods were established for bioassays and field-testing.

Four novel constitutive maize promoters were isolated and Pioneer Hi-Bred filed a U.S. patent application with one Egyptian researcher as a co-inventor. The Provisional Patent Application, *Novel Maize Promoters*, was filed with a priority date of October 6, 1998 and a patent was also filed with the European Patent Office. AGERI will have certain rights to the exploitation of these promoters. This collaboration demonstrates how, through negotiation and collaboration, developing country scientists and institutions can develop and access proprietary innovations.

Through connections made during the project, Pioneer was encouraged to move some insect-resistant maize to test in the Pioneer Hi-Bred breeding program in Egypt. This was a positive impact for Pioneer and for the Egyptian community, since that represented the first testing of such transgenic maize in Egypt.

A strong factor in the success of this project has been that a research agreement was negotiated in the initial stages of project planning that determined ownership and sharing of the IP developed during the project.

Publications

Development of efficient regeneration and transformation system for Egyptian maize inbred lines. A poster presented at the 6th International Conference of Plant Molecular Biology. June 18-24, 2000. Quebec, Canada.

Shireen K. Assem, July (2001): Callus production and plant regeneration in Egyptian maize genotypes. Arab Journal of Biotechnology. July, 2001 (In press)

Shireen K. Assem, Ebtissam H.A. Hussein, Hanaiya A. El-Itriby, Fathy M.Abd El-Galil, and Magdy A. Madkour. (July 2001).The competence of maize shoot meristems for transformation of Egyptian maize inbred lines. Arab Journal of Biotechnology. July, 2001 (In press)

Shireen K. Assem, Hanaiya A. EL-Itriby, Ebtissam H.A. Hussein, Fathy M. Abd El-Galil and Magdy A. Madkour. The regeneration and Transformation of Egyptian maize inbred lines via immature embryo culture and biolistic particle delivery system. Submitted to: In vitro Plant Cellular and Developmental Biology. (May 2001).

F.A. Abdel-Tawab, Hanaiya A. El-Itriby, A. Bahieldin. M.A. Yossef and M.A. Madkour. Regeneration and genetic transformation of different genotypes in maize (Zea mays L.). Egypt. J. of Genet. And Cytol. Vol 30: 1-21, 2001.

El-Itriby H. A., S. K. Assem, E. H. A. Hussein; and M. A. Madkour (2001). The competence of maize shoot meristems for transformation of Egyptian maize inbred lines. Arab Journal of Biotechnology, 4(2): 149-162.

Assem S. K (2001). Callus production and plant regeneration in Egyptian maize genotypes. Arab Journal of Biotechnology, 4(2): 149-162.

CLONING AND CHARACTERIZATION OF INSECTICIDAL GENES FROM BACILLUS THURINGIENSIS.

University of Wyoming, USA
University of Texas at Dallas, USA
AGERI, Egypt

1995-2001

Project Goals

There is increasing concern by scientists, agriculturists and environmentalists about the potential of insects developing resistance to *Bacillus thuringiensis* (Bt) because of its widespread use as an insecticide and in transgenic plants. Bt has been the basis of a variety of biopesticide formulations that have been produced commercially during the past 20-30 years. These biopesticides have been used extensively in the United States and in a number of other countries throughout the world. 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. One insect, the cotton leafworm (*Spodoptera littoralis*) which is a major problem in horticultural crops such as tomatoes, potatoes, and cucurbits as well as in corn, is effectively controlled by Bt insecticidal toxins. Recently, however, the cotton leafworm has exhibited some resistance to Bt toxins. Therefore, it is important to gain a better understanding of the molecular properties of the receptors that bind Bt toxins and that mediate toxicity to insects such as the cotton leafworm. The overall goal of this project is to investigate the molecular basis of insect resistance to the Bt toxins.

Project Activities

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.

POTATO TRANSFORMATION FOR DEVELOPMENT OF TUBER MOTH RESISTANCE

Michigan State University, USA
Agricultural Genetic Engineering and Research Institute (AGERI), Egypt
Central Research Institute for Food Crops (CRIFC), Indonesia
Vegetable and Ornamental Plant Institute (VOPI), South Africa
International Potato Center (CIP), Peru

1995-2001

Project Goal

Potato (*Solanum tuberosum* L.) is an important vegetable crop 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 (PTM) *Phthorimaea operculella* (Zeller). The insect attacks potato plants in two ways: i) by mining the foliage and ii) by feeding on tubers. Therefore, it is an important pest both in field and storage and is currently controlled by large quantities of insecticides applied to the stored tubers. The overall objective of the project is to develop transgenic potatoes with resistance to potato tuber moth.

Project Activities

Researchers at MSU and AGERI have concluded the fourth year of field tests of transgenic potatoes with resistance to Potato Tuber Moth (PTM). The researchers have 3 years worth of data on the resistance of transgenic *Spunta* potatoes, a local fresh market cultivar in Egypt. These *Spunta* lines, transformed with a *Cry5* Bt gene, show strong control of PTM in the tuber (99-100%). Phenotypically, they are similar to untransformed *Spunta*, and should be acceptable to Egyptian consumers and growers. Two years of storage trials have demonstrated that resistance to PTM holds for approximately 2-3 months under ambient storage (using the traditional Nawalla storage system in Egypt) and the results appear long lasting (over a year) in cold storage. MSU researchers currently have additional lines that will be field-tested in early 2001 that will target the Egyptian chip processing industry. 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.

The field tests in Egypt are the most advanced of any trials in the developing world sponsored by the public sector. Future efforts will focus on registration of the materials and developing a resistance management strategy and food safety assessments for the materials in order to commercialize the product. The expertise required for this effort, as well as the costs incurred, are currently being determined. While the research achievements of this project are considerable and are a model for international collaboration in biotechnology, the full impact of this effort will hinge on the difficulty and expense of bringing the transformed lines to the farmers and public. It will be essential to continue to develop a road map for commercialization of products of biotechnology in Egypt, and the potato research provides a perfect platform for this effort.

Project Impacts

- The development of effective gene constructs for potato transformation with Bt genes for resistance to potato tuber moth.

- Development of transgenic potatoes with resistance to PTM. Of particular interest are the newly developed potato lines, Spunta G2 and Spunta G3. These are from a locally grown Egyptian variety transformed with a Cry V vector without the GUS reporter gene and show very high levels of resistance to potato tuber moth. The Spunta variety is locally used and not exported to the EU. It also looks different from the common export varieties, easing any concerns about export problems.
- Multiple years of field-testing of transgenic potatoes in a developing country. Several years of field tests in Egypt and two years of tests with resistant and susceptible tubers in traditional Egyptian storages have been completed. All studies show nearly 100% control of tuber moth, even when the parent Spunta line is heavily infested (up to 100% in storage trials).
- Effective linkages to international centers and to other developing country institutions to expand evaluation of material and to analyze potential impact.
- This has been a very effective project for many reasons. The research team grouping of a plant breeder, a molecular biologist, and an entomologist has been very helpful in taking the research from lab to field.
- There has been a high level of flexibility within the project allowing the researchers to bring other groups and individuals on board as new issues have arisen, e.g. food safety consultancy, and an audit of intellectual property issues.
- Difficulties in the commercialization of these varieties are however anticipated in the regulatory process because the public sector does not have the expertise or the resources to develop such regulatory packages.
- Current transformations are underway using public domain genes that are not under patent, to improve the probability of commercialization.

Publications

J. Cheng, M. Bolyard, R. Saxena and M.B. Sticklen. 1992. Production of insect resistant potato by genetic transformation with a d-endotoxin gene from *Bacillus thuringiensis* var. kurstaki. Plant Science 81(1):83-91.

Reynaldo Ebora and Mariam B. Sticklen. 1994. Resistance of several species of lepidopteran insects to transgenic potato plants. J. Econ. Entomology 87(4):1122-1127

Westedt, A., D. Douches, W. Pett and E. Grafius. 1998. Evaluation of natural and engineered resistance mechanisms in *Solanum tuberosum* for resistance to *Phthormaea operculella* (Lepidoptera: Gelechiidae). J. Econ. Entomol. 91: 552 - 556.

Douches, D.S., A.L. Westedt, K. Zarka, and E.J. Grafius. 1998. Potato transformation to combine natural and engineered resistance for controlling tuber moth. HortScience 33:1053 - 1056.

Li, W., K. Zarka, D. Douches, J. Coombs, W. Pett, and E. Grafius. 1999. Coexpression of potato PVYo coat protein and cryV-Bt genes in potato. J. Amer. Soc. Hort. Sci. 124:218 - 223.

Mohammed, A., D.S. Douches, W. Pett, E. Grafius, J. Coombs, Liswidowati, W. Li, and M.A. Madkour. 2000. Evaluation of potato tuber moth resistance in tubers of *Bt-cry5* transgenic potato lines. J. Econ. Entomology 93: 472 - 476.

Westedt, A., D. Douches, K. Zarka, and E. Grafius. 2001. Transformation with Cry V Bt transgene combined with natural resistance mechanisms for resistance to tuber moth in potato (*Solanum tuberosum* L.). HortScience (in press)

Li, W., D.S. Douches, K.A. Zarka, J.J. Coombs, W.L. Pett, and E.J. Grafius. 2000. Development and evaluation of codon-modified CryV-Bt constructs in cultivated potato, *Solanum tuberosum* L. HortScience (submitted).

Pett, W. L., D. S. Douches, and E. J. Grafius. 1997. Development and field testing of transgenic potatoes with putative resistance to potato tuber moth. In: Japan-U.S.A. Common Agenda. Seminar on seed potato multiplication in Indonesia. Seminar Secretariat: Seed Potato Multiplication and Training Project. Japan International Cooperation Agency. pp1-7.

Douches, D.S., A. Westadt, Liswidowati, A. Hadi-Permadi, W. Pett, and E. Grafius. 1996. Progress in development and evaluation of Bt-transgenic potatoes with resistance to potato tuber moth (*Phthorminaea opurcullela*). Am. Potato J.

Hudy, P., D. Douches, W. Pett, and E. Grafius. 1995. Evaluation of natural resistance, transgenic resistance and the two combined, for control of potato tuber moth (*Phthorminaea opurcullela*). Am. Potato J. 72(10): 639.

Douches, D. S. and W. L. Pett. 1995. Potential environmental concerns *vis-a-vis* the introduction of a specific trait: Experience with potatoes engineered to express insect or virus resistance. In: Environmental concerns with transgenic plants in centers of diversity: Potato as a model. (ed.) R. J. Frederick, I. Virgin and E. Lindarte. The Biotechnology Advisory Commission, Stockholm Environment Institute and Inter-American Institute for Cooperation on Agriculture. pp45-52.

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.

Coombs, J.J., D.S. Douches, W. Li, E.J. Grafius, and W.L. Pett. 2003. Field Evaluation of natural, engineered, and combined resistance mechanisms in potato for control of Colorado potato beetle. J. Amer. Soc. Hort. Sci. 128(2):219-224.

Douches, D.S., W. Pett, F. Santos, J. Coombs, E. Grafius, W. Li, E. A. Metry , M. Madkour and T. Nasr El-Din. 2003. Field and Storage Testing Bt-Potatoes for Resistance to Potato Tuber Moth (Lepidoptera: Gelichiidae). J. Econ. Entomol. (accepted Dec 2002).

Douches, D.S. and E.J. Grafius. 200X. Transformation for Insect Resistance. In Genetic Improvement of Solanaceous Crops, Vol. 2: Potato (Eds. M.K. Razdan and A.K. Mattoo), Science Publishers, Inc. USA (submitted).

DEVELOPMENT OF POTYVIRUS RESISTANT CUCURBITS

Michigan State University, USA
Cornell University, USA
AGERI, Egypt

1995-2001

Project Goal

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. 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. 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. The control of such viruses based on using insecticides and/or inspection and rouging is usually ineffective. The overall goal of this project is to develop high quality cucurbits with multiple virus and disease resistances using a combination of molecular genetic and conventional breeding approaches.

Project Impacts

Cornell University

- A wide array of breeding lines have been developed in 4 cucurbit species widely adapted for tropical and temperate environments with multiple disease resistance.
- In *Cucurbita pepo*, the Eskandarany type favored in the Middle East, caserta, green, grey and black zucchini have been developed with resistance to one or more of the following four viruses, zucchini yellow mosaic virus, watermelon mosaic virus, papaya ringspot virus and cucumber mosaic virus. In all cases, virus resistance has been combined with resistance to a ubiquitous fungal disease, powdery mildew, and in some cases lines have also been bred with reduced attractiveness to cucumber beetles, an important pest, and also vector of bacterial wilt. These lines have been trialed all over the world including Egypt, Jordan, South Africa, the Philippines, Indonesia, and Brazil (see below).
- In *Cucurbita moschata*, the tropical pumpkin, virus resistance has been crossed into tropical types and the plants trialed in the Philippines. Butternut types have been bred with multivirus resistance and resistance to powdery mildew and trialed successfully in South Africa. An open-pollinated variety, Bugle, has been licensed to Seminis for use in South Africa.
- In *Cucumis melo*, resistance has been bred to CMV, PRV, ZYMV and WMV + powdery mildew in sweet orange flesh netted shipper types and sweet crisp green and white flesh melons that combine well as a parent for widespread use in commercial hybrids. Work has also begun to introduce multivirus resistance and powdery mildew resistance to two additional types of tropical melons, Ananas and Galia. With additional support from the American Seed Trade Association, genes have also been identified for high levels of resistance to a fungal disease called Gummy Stem Blight, widespread in the tropics and in humid temperate production areas. Breeding lines have been created with 3 or more of these genes combined to create much higher levels of resistance than observed when the genes are present alone.

- In cucumber, breeding lines have been created in the Beit Alpha (smooth, uniform dark green, glossy, fine spine) and Asian (smooth, very slender and long, uniform dark green, glossy and parthenocarpic) types with various combinations of resistance to the following diseases and pests: four viruses, three leafspots, scab, reduced attractiveness to cucumber beetles, powdery and downy mildew. These breeding lines have been trialed in Egypt, the Philippines, Indonesia, S. Africa, and Brazil.
- This material has also proven useful in the N. American market and in recognition of the importance of these resistances and the product quality of our breeding lines, Jahn and Moriarty were awarded the 2002 Gold Medal for a *C. pepo* variety in the All America Selections/National Garden Bureau competition.
- A major field day was hosted in Ithaca in 2000 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 have been 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.
- Material has also been distributed to a consortium of 27 seed companies from N. America, Europe, Asia, Africa, Australia and New Zealand that are part of the Cornell Vegetable Breeding Institute.

Michigan State University

During the past several years various groups have shown that it is possible to genetically engineer resistance to these viruses in cucurbit crops, but a major limitation to more widespread application of this technology to various cucurbit crops is the lack of efficient transformation systems. For some species there are no available transformation systems, and for others the transformation systems can be very inefficient and/or highly genotype specific. 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 work at MSU is to develop a novel, non-regeneration based system for cucurbit transformation. To this end two approaches are being investigated: one is an electrotransformation system recently developed for use with legume crops. 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. The second approach involves adaptation of a pollen-tube transformation method that has been widely used in China for several species including wheat, cotton, soybean, rice, and recently watermelon. If successful, this method would be even simpler, and involve less sophisticated equipment than electrotransformation.

Results have suggested that the electrotransformation procedure is successful when the DNA is directly incorporated into the developing floral. 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 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. An AGERI researcher currently enrolled as a Ph.D. candidate at MSU has been instrumental in establishing the Agrobacterium-mediated cucumber transformation system and is currently engaged in introducing Arabidopsis cold-responsive transcriptional factor genes to confer resistance to cold, drought or salt.

Egypt

- AGERI researchers, using a construct with the ZYMV coat protein gene developed by MSU, transformed squash plants (using a local Egyptian cultivar, Escandarani) and evaluated resistance under greenhouse and field conditions at AGERI. Preliminary field trials in 1999 and 2000 demonstrated that a majority of transformed plants appeared highly resistant (92-96%) to ZYMV infection, with symptoms of virus infection not appearing until eight weeks post-inoculation.
- Melons have also been transformed to resist ZYMV and these plants have been tested in the greenhouse. AGERI researchers developed a transformation and regeneration system for Shahd EL-Dokki, a local Egyptian cultivar. Two lines were tested through the R_2 generation and a number of plants appeared to be free of virus symptoms at six weeks post inoculation with ZYMV.
- AGERI researchers have introduced the ZYMV coat protein gene into cucumber plants using a local cultivar Beit Alpha via *Agrobacterium tumefaciens* transformation. Four lines contain the ZYMV coat protein gene via ELISA and PCR analysis and await further characterization.
- AGERI researchers have also established a regeneration system in watermelon using the Egyptian cultivars Giza1 and Giza2. This work is still in progress.

Publications

Fang G, Grumet R. 1993. Genetic engineering of potyvirus resistance using constructs derived from the zucchini yellow mosaic virus coat protein gene. Molec. Plant Microbe Interact. 6:358-367.

Yadav RC, Grumet R. 1994. Tendrils as an alternate tissue source for chromosome visualization. J. Amer. Soc. Hort. Sci. 119:850- 852.

Wai T, Grumet R. 1995. Inheritance of watermelon mosaic virus resistance in the cucumber line TMG-1: tissue specific expresssion and relationship to zucchini yellow mosaic virus resistance. Theor. Appl. Genet. 91:699-706.

Wai T, Grumet R. 1995. Inheritance of resistance to the watermelon strain of papaya ringspot virus in the cucumber line TMG-1. HortScience 30:338-340

Grumet R, Yadav RC, Akula G, Hammar S, Provvidenti R. 1995. Genetic engineering of virus resistance in cucurbit crops. Proc.Cucurbitacea '94. Evaluation and enhancement of cucurbit germplasm. Lester GE, Dunlap JR (eds). p. 17-22.

Grumet R, Lanina-Zlatkina T. 1996. Molecular approaches to biological control of virus diseases of plants. In Molecular biology of biological control of pests and diseases of plants.

Gunasekaran M, Weber DJ (eds). CRC Press. p. 15-36. Yadav RC, Saleh MT, Grumet R. 1996. High frequency shoot regeneration from leaf explants of muskmelon. Plant Cell Tiss Org Cult. 45:207-214.

Wai T, Staub J, Kabelka E, Grumet R. 1997. Linkage analysis of potyvirus resistance alleles in cucumber. J. Hered. 88:454-458.

Kabelka E, Grumet R. 1997. Inheritance of resistance to the Moroccan watermelon mosaic virus in the cucumber line TMG-1 and co- segregation with zucchini yellow mosaic virus resistance. Euphytica 95:237-242.

Hokanson SC, Hancock JF, Grumet R. 1997. Direct comparison of pollen-mediated movement of native and engineered genes. Euphytica 96:397-403.

Hokanson SC, Grumet R, Hancock JF. 1997. Effect of border rows and trap/donor ratios on pollen-mediated gene movement. Ecol. Appl. 7:1075-1081.

Kabelka E, Ullah Z, Grumet R. 1997. Multiple alleles for zucchini yellow mosaic virus resistance a the zym locus in cucumber. Theor. Appl. Genet. 95:997-1004

Grumet R, Akula G, Lanina T, Whitaker M. 1998. Transgenic cucurbits: production, uses, and risk assessment. Proceedings XVII Plant Breeding Congress Mexico.

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.

Lecoq H, Dafalla G, Desbiez C, Rip C, DeLecolle B, Lanina T, Ullah Z, Grumet R. 2001. Biological and molecular characterization of Moroccan watermelon mosaic virus and a related potyvirus isolate from Eastern Sudan. Plant Disease. In Press.

Anagnostou, K., M. Jahn and R. Perl-Treves. 2000. Inheritance and linkage analysis of resistance to zucchini yellow mosaic virus, watermelon mosaic virus, papaya ringspot virus and powdery mildew resistance in *Cucumis melo* L. Euphytica 116:265-270.

Zuniga, T., J.P. Jantz, T.A. Zitter and M. Jahn. 1999. Monogenic dominant resistance to gummy stem blight in two melon (*Cucumis melo* L.) accessions. Plant Disease 83:1105-1107.

Silberstein, L., I. Kovalski, R. Huang, K. Anagnostou, M. Jahn and R. Perl-Treves. 1999 Molecular variation in melon (*Cucumis melo* L.) as revealed by RFLP and RAPD markers. Scientia Horticulturae 79:101-111.

Zhang, Y., M.M. Kyle, K. Anagnostou, and T.A. Zitter. 1997. Screening melon (*Cucumis melo* L.) for resistance to gummy stem blight caused by *Didymella bryoniae* in the greenhouse and field. HortScience 32:117-121.

Hoffmann, M.P., R.W. Robinson, M.M. Kyle and J.J. Kirkwyland. 1996. Defoliation and infestation of *Cucurbita pepo* genotypes by diabroticite beetles. HortScience 31:439-442.

Munger, H.M., Y. Zhang, S.L. Fenton and M.M. Kyle. 1995. Leaf blower adapted for large scale inoculation of plants with mechanically-transmitted viruses. HortScience 30:1266-1267.

Gilbert, R.Z., M.M. Kyle, H.M. Munger and S.M. Gray. 1994. Inheritance of resistance to watermelon mosaic virus in *Cucumis melo.* HortSci. 29:107-110.

Brown, R.N., A. Bolanos, J. Myers and M. Jahn. Inheritance of resistance to four cucurbit viruses in Cucurbita moschata. (submitted to Euphytica 12/00).

Kyle, M.M., ed. 1993. Resistance to Viral Diseases of Vegetables: Genetics and Breeding Timber Press, Portland OR.

Jahn, M.M., H.M. Munger and J.M. McCreight. 2001. Breeding cucurbit crops for powdery mildew resistance. In: Powdery Mildews: A compendium. APS Press.

McCouch, S.M., P. Ronald and M.M. Kyle. 1993. Biotechnology and Crop Improvement for Sustainable Agricultural Systems. pp. 157-191. In: Crop Improvement for Sustainable Agricultural Systems. M.B. Callaway and F. Forella, eds. University of Nebraska Press.

Superak, T.H., B.T. Scully, M.M. Kyle and H.M. Munger. 1993. Interspecific Transfer of Viral Resistance. pp. 217-236. In: Resistance to Viral Diseases of Vegetables: Genetics and Breeding. M.M. Kyle, ed. Timber Press, Portland OR.

Munger, H.M., M.M. Kyle and R.W. Robinson. 1992. Cucurbits. pp.42-56. In: Historical Review of Traditional Crop Breeding Practices. Group of National Experts on Safety in Biotechnology Working Group. Directorate for Science Technology and Industry/Committee for Scientific and Technological Policy. Paris: Organization for Economic Cooperation and Development. DSTI/STP/BS (92)5/REV1.

Kyle, M.M. 1995. Breeding cucurbits for multiple disease resistance. Invited paper Cucurbitaceae 94 pp. 55-59 In: Cucurbitaceae 94 (G. Lester and J. Dunlap eds.) Gateway Printing.

Kyle, M.M. 1995. Breeding cucurbits for sustainable production systems. Proc. 1995 NYS Vegetable Conference pp. 22-25.

Zhang, Y., K. Anagnostou, M.M. Kyle and T.A. Zitter. 1995. Seedling screens for resistance to gummy stem blight in squash. Cucurbit Genetics Coop. Rept. 18:59-61.

Kyle, M.M., G. Moriarty and H.M. Munger. 1993. Cucumber, melon and squash germplasm from the Cornell collection. Cucurbit Genetics Coop. Rept. 16:90-91.

Brown, R.N., A. Bolanos, J. Myers and M.M. Jahn. 2003. Inheritance of resistance to four cucurbit viruses in Cucurbita moschata. Euphytica in press.

PRODUCTION OF TOMATO YELLOW LEAF CURL VIRUS (TYLCV) RESISTANT TOMATO

ILTAB, Scripps Research Institute, USA
AGERI, Egypt

1995-1998

Project Goals

Tomato yellow leaf curl disease is a very devastating disease, throughout Africa, the Middle East and South-East Asia. The severity of the disease is dependent on the epidemiology and distribution of the whitefly vector, and as whiteflies are invading new ecological territories, TYLCV is becoming a threat in new areas. Losses to the disease can be extensive and may reach 100% in some areas. In Egypt, of the total production area of 484,963 ha, the losses have been estimated in the range of 5 to 35% from season to season. Current control measures are only partly effective and insecticide treatments are unable to control the vector. The specific objectives of the project were:

1. To establish a collaborative research project with Egyptian counterparts at AGERI, Cairo, Egypt, for exchange of information, reagents, and technology relative to the diagnosis and control of geminivirus diseases in tomato for Egypt.
2. To develop strategies and reagents for the diagnosis of such viruses for use in Egypt and other relevant regions of the world.
3. To develop strategies via plant genetic transformation to develop plants that are resistant to tomato yellow leaf curl virus (TYLCV) a major disease of tomatoes in Egypt.

Project Impacts

- Several clones of TYLCV-Eg were obtained and sequenced by AGERI scientists.
- Oligonucleotide PCR primers were developed that can be used to identify whitefly-transmitted geminiviruses. The primers have been tested at ILTAB and at AGERI, and have been distributed to other researchers around the world for field diagnosis of whitefly-transmitted geminiviruses.
- A large number of chimeric genes were constructed using sequences derived from the genome of TYLCV-Eg.
- Polyclonal antibodies were produced against the coat protein, the pre-coat, and the replicase of TYLCV-Eg. The antibodies for the coat protein are also capable of detecting any whitefly-transmitted geminivirus.
- Tomato transformation has been firmly established with both marker genes and genes derived from the genome of the Egyptian strain of TYLCV. More than 240 transgenic lines were developed. The protocol adopted has led to frequencies of transformation approaching 9% in selected experiments. Transfer of the successful protocol to AGERI has been achieved and tomato transformation can be carried out in Egypt.
- AGERI researchers have identified two different kinds of whitefly-transmitted geminiviruses (tomato yellow leaf curl virus [TYLCV] and tomato yellow mosaic virus [TYMV]) that infect tomatoes in Egypt.
- The genome of Egyptian isolate of TYLCV has been cloned, sequenced and compared with other geminiviruses.
- An infectious TYLCV clone was established and transformed into tomato cultivars that, at the greenhouse level, appear to be resistant to TYLCV infection. The transformed tomatoes carry a cytotoxic gene that is not expressed unless the cell is infected by a whitefly-transmitted

geminivirus. While still preliminary, these early results are among the first demonstrating control of geminivirus.

Using the training received in the U.S. and constructs from U.S. collaborators, AGERI has, to our knowledge, developed the first transgenic tomatoes (and cucurbits – see previous section) within USAID-assisted countries produced by developing country scientists. A number of lines have been field tested at AGERI, and AGERI is currently in active discussions with local industry in how to adapt these materials and/or techniques for the benefit of private sector horticultural interests in Egypt and the Middle East.

Publications

Padidam, M., Beachy, R. N., & Fauquet, C. M. (1995). Classification and identification of geminiviruses using sequence comparisons. Journal of General Virology, 76, 249-263.

Padidam, M., Beachy, R. N. & Fauquet, C. M. (1995b). Tomato leaf curl geminivirus from India has a bipartite genome and coat protein is not essential for infectivity. *Journal of General Virology* 76, 25-35

Padidam, M., Beachy, R. N., & Fauquet, C. M. (1996). Role and function of the AV2 protein of the tomato leaf curl geminivirus. Virology, 224: 390-404.

Padidam, M., Maxwell, D. P. & Fauquet, C. M. (1997). A proposal for naming geminiviruses. *Archives of Virology* 142, 2553-2562.

Padidam, M., Beachy, R., & Fauquet, C. (1998). Bacteriophage M13 ssDNA binding protein complements ssDNA accumulation of tomato leaf curl virus and interferes with viral movement. Submitted to L. Virology.

Padidam, M., Reddy, V. S., Beachy, R. N. & Fauquet, C. M. (1998). Molecular characterization of a plant mitochondrial chaperone GrpE. *Plant Molecular Biology* Submitted.

Padidam, M., Sawyer, S. & Fauquet, C. M. (1998). Recombination is frequent in geminiviruses and may be promoting virus emergence. *Science* Submitted.

Abdallah, N. A., Aref, N. M., Fauquet, C. M., Madkour, M. A., & Beachy, R. N. (1993). Nucleotide sequence and genome organization of an infectious DNA clone of tomato yellow leaf curl virus isolated from Egypt, Glasgow, UK - 8-13 August 1993.

Fauquet, C. M., Padidam, M., & Beachy, R. N. (1994). Sequence comparison of geminiviruses can be helpful for diagnostic and taxonomy. Paper presented at the American Society for Virology - 13th annual meeting, Madison, Wisconsin - 9-13 July 1994.

Fauquet, C. M., Padidam, M., & Beachy, R. N. (1994). Molecular characterization of a tomato leaf curl geminivirus from India. Paper presented at the American Society for Virology - 13th annual meeting, Madison, Wisconsin - 9-13 July 1994.

Fauquet, C. M., Padidam, M., Sangare, A., Deng, D., Fux, C., El-Leithy, S., & Beachy, R. N. (1994). Identification, classification and control of geminiviruses by genetic engineering. Paper presented at the The Sixth Annual Society of Fellows Research Symposium, The Scripps Research Institute, La Jolla, California - 27 May 1994.

Gonzalez de Schöpke, A., Cardenas, L., El Wahed, M., El Bakry, M., Madkour, M. A., Beachy, R. N., & Fauquet, C. M. (1994). *Agrobacterium*-Mediated Transformation of Tomato. Paper presented at the ILTAB Mid-Term Review, TSRI, La Jolla, CA - 28-30 April 1994.

Padidam, M., Beachy, R. N., & Fauquet, C. M. (1994). Tomato Leaf Curl Virus from India (TLCV-In) has a Bipartite Genome. Paper presented at the ILTAB Mid-Term Review, TSRI, La Jolla, California - 28-30 April 1994.

Padidam, M., Beachy, R. N., & Fauquet, C. M. (1994). Identification, Classification and Diagnosis of Geminiviruses. Paper presented at the ILTAB Mid-Term Review, TSRI, La Jolla, California - 28-30 April 1994.

Padidam, M., Beachy, R. N., & Fauquet, C. M. (1994). Comparison of sequences allow precise diagnostic and classification of geminiviruses. Paper presented at the 1st International Symposium on Geminiviruses, Almeria, Spain, 14-17 September 1994.

Padidam, M., Gonzalez de Schöpke, A., El Leithy, S., Abdallah, N., Aref, N., Beachy, R. N., & Fauquet, C. M. (1994). Engineering Resistance against Tomato Yellow Leaf Curl Virus (TYLCV)-Egypt. Paper presented at the ILTAB Mid-Term Review, TSRI, La Jolla, California - 28-30 April 1994.

Beachy, R. N., Padidam, M., Sangare, A., & Fauquet, C. M. (1995). Viral pathogenesis and pathogen derived resistance: expectations and limitations for control of geminiviruses. Paper presented at the USA-Mexico Symposium, Cocoyoc, Mexico, 5-9 November 1995.

Padidam, M., Beachy, R. N., & Fauquet, C. M. (1995). AV2 ("Pre Coat) protein of tomato leaf curl virus - India (ToLCV-In) is required for normal levels of viral DNA accumulation and symptom development. Paper presented at the Biology and Molecular Epidemiology of Geminiviruses Symposium, Westward Look Resort, Tucson, Arizona - 3-8 June 1995.

Gonzalez de Schopke, A., Beachy, R. N., & Fauquet, C. M. (1996). *Agrobacterium*-mediated transformation in tomato. Paper presented at the ILTAB Review 1991-1996, TSRI, La Jolla, CA. - 21-22 March 1996.

Padidam, M., Beachy, R. N., & Fauquet, C. M. (1996). Classification and identification of geminiviruses. Paper presented at the ILTAB Review 1991-1996, TSRI, La Jolla, CA. - 21-22 March 1996.

Padidam, M., Beachy, R. N., & Fauquet, C. M. (1996). AV2 protein is required for efficient viral movement and coat protein regulates ssDNA accumulation in tomato leaf curl virus. Paper presented at the ILTAB Review 1991-1996, TSRI, La Jolla, CA. - 21-22 March 1996.

Padidam, M., Beachy, R. N. & Fauquet, C. M. (1994). Comparison of sequences allow precise diagnostic and classification of geminiviruses. In *1st International Symposium on Geminiviruses*. Almeria, Spain, 14-17 September 1994.

Padidam, M., Beachy, R. N. & Fauquet, C. M. (1997). Production of resistant plants to geminiviruses by expressing mutated pre-coat proteins. In *ILTAB/TSRI-ORSTOM*. USA.

Padidam, M., Chatterji, A., Beachy, R. N. & Fauquet, C. M. (1998). Role of AV2, BV1 and BC1 proteins in tomato leaf curl virus movement. In *2nd International Workshop on Bemisia and Geminiviral Diseases*. San Juan, Puerto Rico - 7 to 12 June 1998.

Padidam, M., Sawyer, S. & Fauquet, C. M. (1998). Genetic recombination is frequent among geminiviruses. In *2nd International Workshop on Bemisia and Geminiviral Diseases*. San Juan, Puerto Rico - 7 to 12 June 1998.

WHITEFLY BIOTYPES AND BIOTYPE-SPECIFIC TRANSMISSION OF GEMINIVIRUSES

University of Arizona
AGERI, Egypt

1995-1998

Project Goals

Whitefly-transmitted geminiviruses are among the most important emerging viral pathogens in arid, irrigated monoculture systems, worldwide. Geminiviruses have emerged as global pathogens due to recent upsurges in populations of *Bemisia tabaci,* the only known vector of this group of geminiviruses. Little is known about the identity and distribution of geminiviruses infecting vegetable crops in Egypt, and there is no information concerning geminivirus-whitefly vector interactions except one study conducted at AGERI under this project. Populations or biotypes of the most important geminivirus vector, *Bemisia tabaci* (Genn.), are morphologically indistinguishable, yet they have adapted to distinct niches in native and cultivated plant communities and are highly variable in terms of their actual threat to crops.

Different populations of *B. tabaci* vary with respect to biological attributes such as host preferences, virus vector capacities, and levels of insecticide resistance. Accurate biotyping of whiteflies is therefore important for implementing effective crop management strategies to control virus diseases transmitted by whiteflies and the damage inflicted by whitefly feeding. Specific objectives of this project were as follows:

- To identify the distinct whitefly vector populations in tomato and vegetables using protein polymorphism and molecular markers.
- To define population-specific transmission profiles between predominant whitefly biotypes and TYLCV in tomato, and other geminiviruses of vegetable crops.

In order to achieve these goals the specific research objectives were as follows:

1. To identify primers that will differentiate between Egyptian populations of *Bemisia tabaci,* the vector of tomato yellow leaf curl, collected from isolated geographic locales in crop and weed hosts of the whitefly,

2. To investigate the degree of relatedness between Egyptian whitefly vector populations and those in the adjacent regions,

3. To corroborate biological (host preferences, vector relations), biochemical, and molecular data sets for populations studied in the AZ and AGERI laboratories.

Project Impacts

- Documentation of the distribution of distinct whitefly vector populations in Egypt using biochemical and molecular markers in collaboration with AGERI scientists.
- Initiation of tracking of distinct vector populations and geminiviruses associated with vegetable crops, particularly tomato, throughout Egypt and within the region.
- Establishment of whitefly vector colonies at AGERI and whitefly-transmission experiments with tomato yellow leaf curl virus using two whitefly species, *B. tabaci* and *T. ricini*. Substantial work was conducted with *T. ricini*.

- The AZ group developed the core Cp primers for the detection of geminivirus coat protein gene fragment in single whitefly vectors and in plant samples.
- PCR primers from AZ and AGERI laboratories are now available to detect geminiviruses in individual whiteflies and infected plants.

DEVELOPING DROUGHT AND SALINITY TOLERANT WHEAT AND TOMATO FOR EGYPTIAN AGRICULTURE

Ohio State University, USA
AGERI, Egypt

1998-2001

Project Goal

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. Agricultural development in many areas of the country 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 (strategic commodities in the Middle East) makes it imperative to increase cultivation in the areas where sub optimal conditions, such as water deficit, salinity, and high temperature, prevail.

The overall goal of this project is to enhance osmotic stress tolerance in Egyptian wheat and tomato crops. This will be achieved by over expressing the key regulatory enzymes of the proline biosynthesis and sulfur assimilation pathways. Research will investigate whether elevated levels of proline and active sulfur confer drought and salinity tolerance in two plant systems, i.e., wheat and tomato, and attempts will be made to find gene(s) able to convert proline into proline betaine.

Project Progress

AGERI scientists have established a transformation and regeneration system for wheat and transformed a number of genes that have been reported to affect drought and salt tolerance. The *mtlD* gene (from *E. coli* and which accumulates mannitol), the HVA1 gene (from barley and which confers delayed leaf wilting), and the fructan gene (from *Bacillus subtilis* and which plays a role in osmotic adjustment to changing environmental conditions) were all transformed into wheat. Early results indicate that the transformed lines are expressing the genes and proteins and, under laboratory conditions, appear to be more salt tolerant than controls. Confirmation of these results await greenhouse and field tests.

FINAL RESEARCH REPORTS

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

Principal Investigators:

Rebecca Grumet, Michigan State University

Molly Jahn, Cornell University

Project Partners:

Dr. Atek Sadik, AGERI, Egypt

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

Objectives

The primary objective of the cucurbit subproject was to develop virus resistant cucurbit crops using a combination of molecular genetic and conventional breeding approaches. This effort included an endeavor to develop an effective transformation system for cucurbits. Most recently we also introduced genes into cucumber that may confer salinity and drought tolerance.

Importance of the problem and rationale for approach

Cucurbit species include a variety of high value crops (e.g., melons, watermelon, cucumber, summer squashes, winter squashes) that play important roles in both local diets and as export crops throughout the world. Within Egypt, 46,000 Ha of watermelons, 28,000 Ha of squash, pumpkins and gourds, 20,000 Ha of cantaloupes and other melons, and 18,000 Ha of cucumbers are produced annually. A major limitation of successful production of these crops is infection by viruses including several potyviruses such as zucchini yellow mosaic virus (ZYMV) and the cucumovirus, cucumber mosiac virus. Productivity is also limited by environmental dehydration-related stresses such as drought and salinity.

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, was produced commercially. A major limitation to more widespread use of this technology for various cucurbit crops, or improvement of cucurbit crops in general, is lack of efficient transformation systems. For several species there are no available transformation systems; for other species the transformation systems can be very inefficient and/or highly genotype specific. Often the difficulty in developing effective transformation systems lies in the tissue culture based process that requires successful regeneration from individual cells. One objective of the project was to establish an efficient cucumber transformation system, with emphasis on the development of a novel, non-regeneration based system. Cucumber was chosen as the crop of focus because of its importance in Egypt, interest by our AGERI collaborators, and poor efficiency of then available cucumber transformation systems.

Salinity and drought stress experiments were initiated with the development of the effective cucumber transformation system and availability of novel genes developed by MSU scientists, the Arabidopsis *CBF*

(C-repeat binding factor) and celery mannose-6-phosphate reductase (M6PR). The CBF transcription factor is associated with increased freezing, drought, and salt stress by inducing expression of a number of genes that allow for the stress tolerance response (Thomashow, 1999). Similarly, introduction of the M6PR gene into Arabidopsis caused increased salt stress tolerance by allowing for enhanced osmotic adjustment (Gao and Loescher, 2002).

Description of Activities:

I. Produce melon genotypes that express the ZYMV-CP gene; characterize the nature and extent of resistance and perform field trials.

The initial ZYMV-CP mediated resistance work had been done with an American cultivar, Hale's Best Jumbo, based on its responsiveness in tissue culture (Fang and Grumet, 1993). An initial priority was to introduce the ZYMV-CP gene into melon cultivars suitable for production conditions in Egypt. To this end, we introduced the ZYMV-CP gene into the Egyptian melon cultivars 'Shahd El Dokki' and 'Ananas El Dokki' and the American cultivar 'Topmark'. Successful transformation of all three genotypes was verified by expression of the introduced marker gene and presence of the ZYMV CP gene by PCR analysis. Transgenic materials were tested for response to inoculation with ZYMV (Fig. 1; Grumet et al. 1995). Performance of the diploid lines was variable, the most promising lines were propagated for seed production in the greenhouse. Field trials were performed to test several promising transgenic lines (R_2 and R_3 generation), hybrids between transgenic 'Hale's Best Jumbo' and Egyptian cultivars, and various parental controls. Each genotype was examined for growth, yield, and response to viral inoculation in a split plot design with three replications (Fig 1). Segregating progeny of two transgenic lines had a long delay in infection (> 45 days) or did not become infected. Viral inoculation caused yield reductions of 33%- 60% for the parental genotypes ('Hale's' and 'Topmark', 33%; 'Ananas', 60%). We were unable to judge the response of 'Shahd', it did not produce fruit in our conditions. Our best R_2 and R_3 transgenic lines had 0 - 20% yield reductions.

Figure 1. Greenhouse and field testing of transgenic Ananas and Shahd lines transformed with the ZYMV-CP gene for response to ZYMV infection.

Collaborations between MSU and AGERI led to the transfer of melon transformation technologies and the ZYMV coat protein gene to AGERI. Scientists at AGERI successfully used this gene to produce transgenic melons and squash and have performed virus testing in the greenhouse and field. Egyptian field trials performed by the AGERI collaborators verified that the ZYMV-Ct coat protein gene was able to confer resistance to the Egyptian strain of ZYMV.

II. Cucurbit transformation.

A. Non-regeneration based approaches.

Two non-regeneration based approaches were tested: electrotransformation and pollen-tube mediated transformation. Electrotransformation was originally developed by Dr. Richard Allison (Michigan State University) for legume crops. Pollen tube transformation has been reported in China, but has received little attention in Western laboratories. Our initial goals for the electrotransformation procedure were to determine the parameters appropriate for cucumber including appropriate stage of seedling development, handling procedures to insure transformation and recovery, and the appropriate electrotransformation settings. Although analysis of vegetative tissue plants indicated a PCR-positive response for approximately 10% of the plants, we did not observe transfer of the gene to the next generation. The most likely explanation for the discrepancy between the vegetative tissue and the progeny was that the original treated plants were chimeric (i.e., containing a mixture of transformed and non-transformed tissue) and that the floral tissue had not been transformed. The electrotransformation system was then refined to utilize older seedlings at time when they are in the process of initiating floral primordia (7-10 days; Goffinet, 1990), and switching to a gynoecious breeding line which would allow for fruit set at the earliest nodes. Flowers were pollinated at the first four flowering nodes of the gynoecious electro-treated seedlings and fruit collected from approximately 200 plants. We did not obtain stable transgenic plants using this method.

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

B. Agrobacterium mediated transformation:

1. Melon tissue culture and transformation experiments. An efficient melon leaf regeneration system was developed tested for suitability for *Agrobacterium* mediated transformation (Fig. 2; Yadav et al. 1996). This work was a collaboration between MSU and visiting AGERI scientists. The leaf-regeneration system was demonstrated to be useful for transformation; PCR-verified transgenic shoots and plants were produced using this method. This method is especially useful for genotypes with limited seed supplies (e.g., experimental breeding lines).

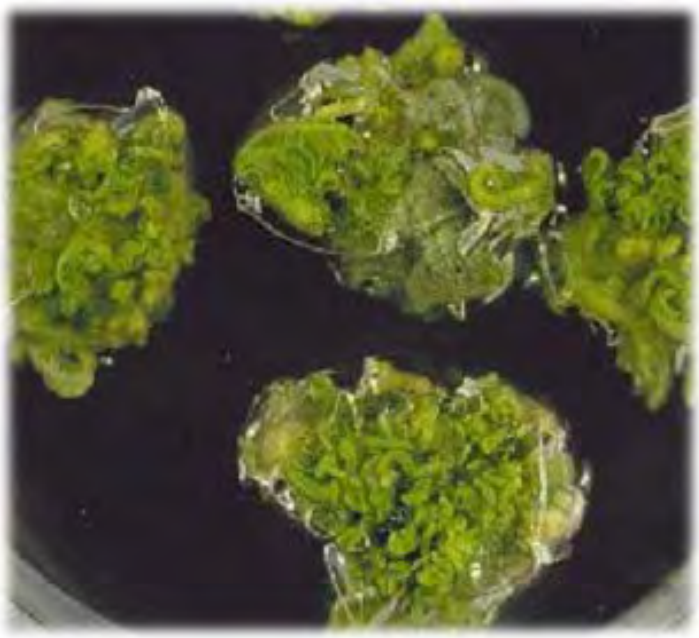


Figure 2. Development of an efficient leaf regeneration method for melon. This method was used successfully to produce transgenic melon plants using *Agrobacterium*-mediated transformation.

2. Agrobacterium-mediated transformation of cucumber. Agrobacterium-mediated transformation of cucumber has become increasingly efficient in the laboratory and we have introduced at least seven different gene constructs into monoecious (Straight 8) and gynoecious (GP14) genotypes. Mohamed Tawfik, an Egyptian Ph.D. student from AGERI has introduced the Arabidopsis *CBF* (C-repeat binding factor) and celery mannose-6-phosphate reductase (M6PR) dehyration stress-related genes into cucumber. The CBF transcription factor is associated with increased freezing, drought, and salt stress by inducing expression of a number of genes that allow for the stress tolerance response (Thomashow, 1999). Similarly, introduction of the M6PR gene into Arabidopsis caused increased salt stress tolerance (Gao and Loescher, 2002).

Successful transfer of the *CBF* gene has been verified by PCR analysis of 14 primary regenerants and expression verified for these individuals by northern analysis (Tawfik and Grumet 2001). T1 seed was produced from 13 T0 individuals. Transfer of the introduced gene to the next generation has been verified for ten T1 lines; the ca. 3:1 ratios of PCR+/PCR- plants for these families are consistent with single gene integration (Table 1).

Table 1. Transfer of introduced *Arabidopsis CBF* genes into cucumber as verified by transgenic progeny.

Line	Construct	Transgenic: Non-transgenic	X^2
A1	CBF1	46:11	0.707 ns
A3		49:13	0.301 ns
A4		45:13	0.092 ns
A5		43:12	0.151 ns
A6		47:16	0.005 ns
B1	CBF3	44:12	0.215 ns
B4		46:10	1.141 ns
B5		49:10	1.632 ns
B6		47:9	0.690 ns
B7		40:12	0.025 ns

In summary, electrotransformation and pollen-tube mediated technologies gave sporadically positive, but non-reproducible results. However, we were able to establish an effective *Agrobacterium*-mediated transformation system for cucumber and have successfully introduced several genes of interest.

III. Salinity testing of transgenic cucumber lines.

Transgenic progeny from ten T1 lines transformed with CBF genes were subjected to salt stress treatments in the greenhouse (Fig. 3). The transgenic individuals showed significantly higher total sugars (2-3 fold) and proline content (2-3 fold) than did control, non-transgenic plants and non-transgenic siblings from the transgenic families (Analysis of variance $P<0.001$) (Fig. 4). This difference was further enhanced in the plants subjected to treatment with 100 mM NaCl. Equivalent results regarding were obtained in two experiments. Presence of the CBF gene also had a positive effect on dry weight accumulation under the salt stress conditions. Under non-salt conditions, the control and transgenic lines showed varying, but similar growth; dry weight was not significantly different between the controls and transgenics. When treated with 100 mM salt, however, the two controls showed a significant decline in dry weight accumulation while the transgenics showed little or no decrease. Dry weight accumulation in salt stress conditions was directly correlated with the total sugar content of the plants; the transgenic plants which exhibited higher sugar levels showed higher dry weight ($r=0.862$).

These results indicate that presence of the *CBF* gene caused physiological adjustments associated with dehydration stress responses and that *CBF* may help to reduce negative growth effects caused by salt stress.

Fig. 3. Salt stress experiments in the greenhouse with transgenic CBF-cucumbers.

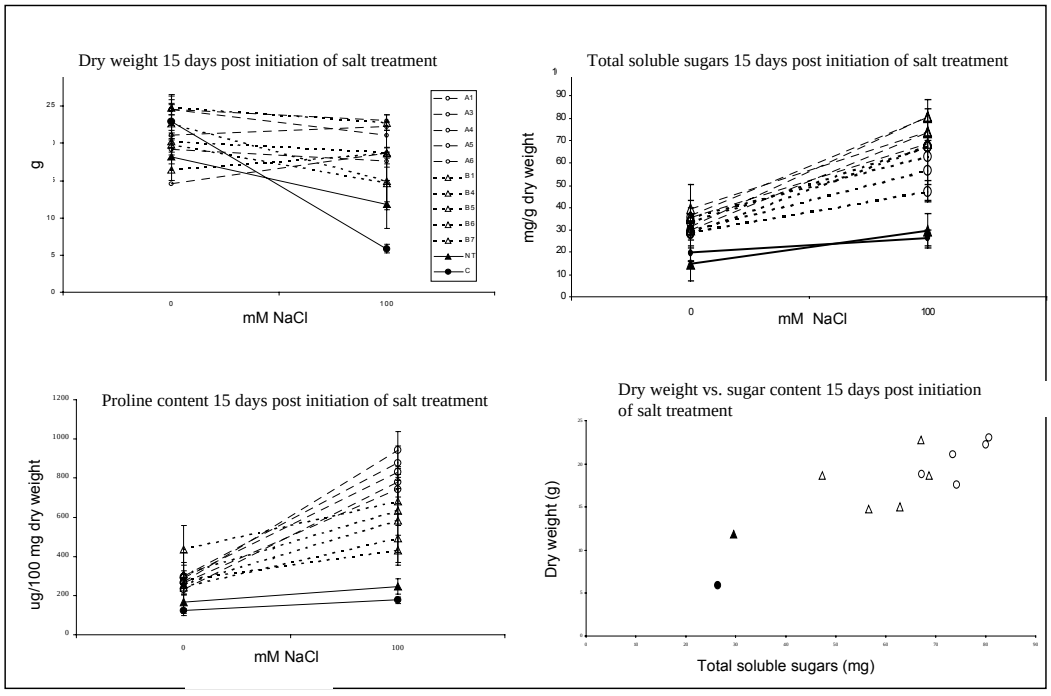


Figure 4. Response of transgenic cucumbers transformed with CBF1 or CBF3 genes to treatment with 100 mM NaCl. Each point is the mean of six replicate plants ± S.E.

IV. Risk assessment.

An additional project for which the ABSP program contributed a limited portion of funding, was directed toward risk assessment due to transgenic pollen flow from cucurbits. Our studies focused on two questions: can plantings of border rows effectively limit pollen mediated gene movement, and does pollen-mediated dispersal of transgenes differ from native genes? As the trap/donor ratio increased, there was a signficant decrease in long distance movement to satellite plots; however to prevent gene movement, even from small experimental plots, would require excessive non-transgenic trap plants, indicating that even in small plot trials, there will be gene escape (Hokanson et al. 1997a). With regard to the question, does pollen-mediated dispersal of transgenes differ from native genes, short and long distance gene movment data validate the assumption that native and transgenes have the same dispersal patterns (Hokanson et al. 1997b). Reviewer's responses reflected our intended goal: "although almost anyone in the field would have predicted that pollen containing a transgene would have the same pattern of dispersal as non-engineered...this is a well done study that proves a common assumption in a clear and definite way...for everyone's benefit it is very important that this issue be seriously addressed and that regulatory guidelines be developed on fact rather than assumption".

Literature Cited

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.

Gao Z, Loescher W. 2002. Expression of a celery mannose-6-phosphate reductase in Arabiodpsis thaliana enhances salt tolerance and induces biosynthesis of both mannitol and a glucosyl-mannitol dimer. Plant Cell Env. In press.

Grumet R, Yadav RC, Akula G, Hammar S, Provvidenti R. 1995. Genetic engineering of virus resistance in cucurbit crops. Proceedings Cucurbitaceae: Evaluation and enhancement of cucurbit germplasm. Lester GE, Dunlap JR (eds). p. 17-22. (Invited)

Hokanson SC, Grumet R, Hancock JF. 1997a. Effect of border rows and trap/donor ratios on pollen-mediated gene movement. Ecological Applications. 7:1075-1081.

Hokanson SC, Hancock JF, Grumet R. 1997b. Direct comparison of pollen-mediated movement of native and engineered genes. Euphytica. 96:397-403.

Tawfik MS, Grumet R. 2001. Production of transgenic cucumber plants expressing the Arabidopsis cold stress-related transcription factors, CBF1 and CBF3. HortScience 36:455 (abstract).

Thomashow MF. 1999. Plant cold acclimation: fresszing tolerance genes and regulatory mechanisms. Annu. Rev. Plant Phys. Mol. Biol. 50:571-599.

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

Publications citing ABSP support during the reporting period

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

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

Tawfik MS, Grumet R. 2001. Production of transgenic cucumber plants expressing the Arabidopsis cold stress-related transcription factors, CBF1 and CBF3. HortScience 36:455 (abstract).

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.

Grumet R, Akula G, Lanina-Zlatkina T, Whitaker M. 1998. Transgenic cucurbits: production, uses, and risk assessment. Procedings XVII Plant Breeding Congress, Mexico. (Invited)

Grumet R, Kabelka E, McQueen S, Humphrey R. 1998. Characterization of sources of resistance to the watermelon strain of papaya ringspot virus in cucumber: allelism and co-segregation with other potyvirus resistances. Cucurbitaceae '98. In press.

Grumet R, Akula G, Laninna-Zlatkina T, Whitaker M. 1998. Transgenic cucurbits: production, uses and risk assessment. Proceedings XVII Plant Breeding Congresss, Mexico.

Hokanson SC, Hancock JF, Grumet R. 1997. Direct comparison of pollen-mediated movement of native and engineered genes. Euphytica. 96:397-403.

Hokanson SC, Grumet R, Hancock JF. 1997. Effect of border rows and trap/donor ratios on pollen-mediated gene movement. Ecological Applications. 7:1075-1081.

Kabelka E, Ullah Z, Grumet R. 1997. Multiple alleles for zucchini yellow mosaic virus resistance at the zym locus in cucumber. Theor. Appl. Genet. 95:997-1004.

Kabelka E, Grumet R. 1997. Inheritance of resistance to the Moroccan watermelon mosaic virus in the cucumber line TMG-1 and cosegregation with zucchini yellow mosaic virus resistance. Euphytica 95:237-242.

Wai T, Staub JE, Kabelka E, Grumet R. 1997. Linkage analysis of potyvirus resistance alleles in cucumber. J Hered. 88:454-458.

Grumet R, Lanina-Zlatkina T. 1996. Molecular approaches to biological control of virus diseases of plants. In Gunasekaran M, Weber DJ (eds). Molecular biology of biological control of pests and diseases of plants. CRC Press. p. 15-36. (Invited)

Yadav RC, Saleh MT, Grumet R. 1996. High frequency shoot regeneration from leaf explants of muskmelon (Cucumis melo L.). Plant Cell Tiss. Org. Cult. 45:207-214.

Hancock JF, Grumet R, Hokanson SC. 1996. The opportunity for escape of engineered genes from transgenic crops. HortScience. 31:1080-1085.

Grumet R, Yadav RC, Akula G, Hammar S, Provvidenti R. 1995. Genetic engineering of virus resistance in cucurbit crops. Proceedings Cucurbitaceae: Evaluation and enhancement of cucurbit germplasm. Lester GE, Dunlap JR (eds). p. 17-22. (Invited)

Grumet, R. 1995. Genetic engineering for crop virus resistance. HortScience 30:449-456. (Invited).

Grumet R. 1995. Coat protein mediated virus resistance in plants. In: Transgenic virus resistant plants and new plant viruses. Ameican Institute for Biological Sciences. Washington DC. P.17-20. (Invited)

Wai, T. and Grumet, R. 1995. Inheritance of resistance to the watermelon strain of papaya ringspot virus in the cucumber line TMG-1. HortScience 30:338-340.

Wai, T. and Grumet, R. 1995. Inheritance of watermelon mosiac virus resistance in the cucumber line TMG-1: tissue specific expression and relationship to zucchini yellow mosaic virus resistance. Theor. Appl. Genet. 91:699-706.

Field Testing of Disease -Resistant Cucurbit Crops and Transfer of Improved Publicly Developed Breeding Lines to Private Companies and Public Agencies in the Developing World

Principal Investigator

Molly Jahn, Cornell University

Other Principal Investigators during the funding period

Henry M. Munger and R. Provvidenti

Summary

If the benefits of biotechnology beyond the major commodity crops are ever to be broadly distributed in agricultural fields in the developing world, procedures and capacities to transfer useful germplasm to breeding programs around the world must be in place. Simple mechanisms must be developed to transfer improved germplasm and associated intellectual property to the targeted recipient, and assist the recipient in the necessary evaluations of the material and necessary risk assessment required to move the germplasm towards improved adapated varieties. Finally, an appropriate seed distribution system must be in place so that good quality seed that is true to type is available widely to farmers. We have stressed private sector partnerships where possible, as one way to efficiently address downstream issues.

This component of ABSP had two primary goals. The first was to generate improved germplasm as the foundation for transgenic efforts in Rebecca Grumet's group aimed to enhance specific resistances and also for use directly in the developing world. The second was to establish the linkages necessary to deliver genetically improved cucurbit crops to public and private sectors in selected countries in the developing world. Initially, our work focused on Egypt, but in the last 2 years of the project, we expanded our targets to include Jordan, South Africa, Indonesia, the Phillipines, and Brasil. The primary technical focus was on virus resistance in the cucurbit crops, namely squash, melons and cucumber, but we requested and received permission to address a total disease resistance package necessary to achieve beneficial impacts on yields. The rationale was to establish linkages and evaluation procedures with conventionally bred germplasm due to the relative simplicity of this process, and then build upon these bridges to transfer GMOs with the additional IP, evaluation and risk assessment requirements.

This subproject was extremely successful in setting up linkages in the developing world with both public (Egypt) and private sector players (Brasil, Egypt, Indonesia, Phillipines, Pakistan, South Africa). Germplasm has been transferred under material transfer agreements to the following regional/national seed companies: Hytech (Egypt), East West Seeds (Phillipines, Thailand, Indonesia), North South Seeds (Pakistan), Pannar and Alpha Seeds (South Africa), Agriflora (Brazil). We have commercialized three varieties in Brazil, one in S. Africa, one in Pakistan and several varieties are near release in other regions in Asia where we have only recently focused our efforts that should have a dramatic effect on stability of yields and product quality. In general we were less successful with the public sector. Our materials were included in several field trials in Egypt where they showed good resistance, but the materials were not aggressively pursued for variety development or for use in transformation. Similarly, while we were able

to provide the MSU team with many different types of starting materials for transformation, none served as the base for transformation efforts that yielded agriculturally useful germplasm.

In some cases, germplasm transfers to companies in the developing world were straightforward because of existing relationships and relatively sophisticated counterparts, but in other cases, extensive negotiations were necessary before the company would accept the standard material transfer agreement we use. In general, considerable technical assistance was also provided to support in-country evaluation efforts including the sharing of inoculation and evaluation protocols, assistance in interpreting results, coordination of our screening with work done in developing countries, etc. As a consequence of these efforts, in several cases, we were able to implement license agreements successfully under standard terms, and we anticipate that additional licenses will be signed as the germplasm moves forward into regional trials.

The investment made in this breeding program will continue to provide benefits through a large consortium of seed companies that have now banded together to provide continuing funding in the form of unrestricted gifts provided by each member on a sliding scale for this effort. ABSP allowed the smaller regional companies to become members of this group, and in the case of Alpha Seeds and East West Indonesia, actually supported the attendence of key plant breeders to the Cornell Vegetable Breeding Field Days, which greatly facilitated our collaborations and evaluations.

A complete list of the specific products of our work supported by this subcontract include breeding lines and varieties with multiple disease resisance in the following crop types: Eskandarany and a wide range of other summer squash types , orange and green flesh melon, and Asian and Middle Eastern cucumbers. In some regions of the developing world, a second squash species, *C. moschata*, is eaten either immature or in its mature form (pumpkin, a distinct species from the jack o'lantern pumpkin used in N. America). We also have breeding lines in these types for Brazil and S. Africa. The diseases we have bred to control are as follows: cucumber mosaic virus, papaya ringspot virus, watermelon mosaic virus, zucchini yellow mosaic virus, powdery and downy mildew, 4 cucumber leafspot diseases caused by fungal pathogens (*Alternaria*, Anthracnose, *Corynespora*, *Ulocladium*), melon and squash gummy stem blight.

Two of our sites were the focus of an intensive socioeconomic analyses and in both cases, although the study was conducted at sites for which we had been breeding just a very short time (South Africa and Indonesia), the investment in multiple disease resistant cucurbit varieties was demonstrated to be a very efficient expenditure for poverty alleviation and private sector expansion.

No equipment was purchased using these funds. The funds provided to us were used to pay staff and seasonal crews, cover the costs of greenhouse and field-based research and laboratory-based support of these efforts. Tammy Thomas will provide final receipts and budget reports.

Maize Transformation for Development of Stem Borer Resistance

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

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 *0. nubilalis* is extended. *C. agamemnon* has an economic threshold similar to that of *O. nubi1alis* 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 zyogotic 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 a1, 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 is 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).

Future Work

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.

Whitefly Biotypes and Biotype-Specific Transmission Of Geminiviruses In Egypt And Arizona

Principal Investigator

Dr. Judith K. Brown, University of Arizona

US Research Team (university of Arizona)

Dr. Judith Brown (PI)

Dr. A. M. Idris (Post-Doctoral Associate)

Mr. Rafael Caballero (Ph.D. student)

Ms. Ivone Torres-Jerez (Research Specialist)

Ms. Betsy Lewis (Laboratory Technician)

Summary

Accurate biotyping of whiteflies is essential for implementing effective crop management strategies to control virus diseases transmitted by whiteflies and damage inflicted by whitefly feeding alone, because different populations of *B. tabaci* vary with respect to biological attributes such as host preferences, virus vector capacities, and levels of insecticide resistance. To develop a rapid and accurate method for identifying whiteflies in general, and to distinguish between members of the *B. tabaci* species complex, we applied a molecular marker approach to facilitate whitefly identification by DNA sequence comparison with well-characterized, reference whitefly populations to investigate whitefly relationships based upon molecular sequences that might be useful for identifying *B. tabaci* and its close relatives using DNA based technologies. Further, sequence comparisons can yield inferred evolutionary histories from which predictions can be made concerning the origin of a particular whitefly population. A molecular sequence serves as a signature sequence for each population that can aid in tracking a particularly troublesome pest or vector within mixtures of biotypes, and can alert to an introduction or emergence of a new pest population. PCR-based technology was also developed and applied. The AZ developed the core Cp primers for the detection of geminivirus coat protein gene fragment of in single whitefly vectors and in plant samples. Gene fragments can be sequenced to confirm or determine the identity of the particular virus. Virus transmission profiles were examined for tomato yellow leaf curl virus and the whitefly, *T. ricini* , in the AGERI laboratory. PCR primers from AZ and AGERI laboratories are now available to detect geminiviruses in individual whiteflies and infected plants.

Project Background

Whitefly-transmitted geminiviruses (Subgroup III, Geminiviridae) are among the most important emerging viral pathogens in arid, irrigated monoculture systems, worldwide. Geminiviruses have emerged as global pathogens due to recent, upsurges in populations of *Bemisia tabaci*, the only known vector of subgroup III geminiviruses. Little is known about the identity and distribution of geminiviruses infecting vegetable crops in Egypt, with the exception of research conducted recently by AGERI scientists on tomato yellow leaf curl virus. Further, there is no information concerning geminivirus-whitefly vector interactions except one study conducted at AGERI under this project.

Whiteflies are Homopteran insects that transmit plant-infecting viruses to a wide range of vegetable crops, yet it is nearly impossible to accurately identify whiteflies within the same genus, species, or subspecies using classical morphological methods. Further, populations or biotypes of the most important geminivirus vector, *Bemisia tabaci* (Genn.), are morphologically indistinguishable, yet they have adapted to distinct niches in native and cultivated plant communities and are highly variable in terms of their actual threat to crops. The fear of the damage as a pest and virus vector by the most threatening members of the *B. tabaci* complex has led to the rampant and indiscriminant use of pesticides, often against populations that may not pose an economic threat to the crop. Consequently, natural enemies are often annihilated and insecticide resistance develops in the more threatening biotypes. In conjunction with unpredictable upsurges in whitefly population levels, geminivirus-incited diseases subsequently reach epidemic proportion in a region. A single infiltration of virus inoculum into crops and weeds in a region often results in establishment of a permanent baseline level of geminivirus pathogens that serves as the source of inoculum for subsequent crops. To date, there are no disease resistant vegetable varieties available for Egyptian conditions. Clearly, accurate identification of whitefly vector populations and the geminiviruses they transmit are essential to achieving effective targeting of control strategies including chemically-based and integrated pest management approaches.

Recent research efforts in the AZ laboratory have focused on defining the extent of variation between *B. tabaci* populations, and how particular variants impact crop productivity as both pests and as vectors of geminiviruses. We previously examined biological, morphological, and biochemical differences between representative populations of *B. tabaci* collected from different hosts and geographic regions (Bedford et al., 1995; Brown et al., 1995a,b; Costa and Brown, 1991; Costa et al., 1993; Rosell et al., 1998). Collectively, studies have revealed a high degree of genetic and biological variation which led to the proposed recognition of *B. tabaci* as a species complex (Brown et al., 1995b). Recent knowledge of substantial biological variation within this species has reinforced the need to examine the differential impact on crop production and on control programs established to reduce *B. tabaci* pest and vector populations.

This project was initiated to develop and apply molecular based approaches to identifying and tracking whiteflies important in vegetable crops in Egypt, and to permit a systematic evaluation of whitefly and geminivirus identity and distribution in agroecosytems over time. To expand the COI data base required for accurate identification of whiteflies in the region, the COI marker was obtained from additional populations representing adjacent and distant geographic sites and their sequences were obtained. Whitefly COI sequences will be complied in an interactive comparative DNA sequence database that will be accessible to all on the World Wide Web. Users may PCR-amplify the target region of the marker gene, obtain its DNA sequence, and compare it interactively to sequences available at the site. Other relevant information about biological characters and distribution of distinct populations will be accessible at the site. This is the first effort of its kind to facilitate accurate identification of *B. tabaci* , a requisite to rationale implementation of strategies toward control of whiteflies as pests and vectors of geminivirus-incited diseases. Another aspect of the project involved the application of PCR-based detection of geminiviruses in individual whitefly vectors to track the distribution of vector populations and associated viruses. A second aspect of this objective was to establish transmission profiles for select Egyptian vector biotypes and tomato yellow leaf curl virus, using PCR detection to monitor virus ingestion by whiteflies.

Project Goals

1. *To identify the distinct whitefly vector populations in tomato and vegetables using protein (non-specific esterase profiles) polymorphism and molecular markers (mitochondria 16S rRNA and COI genes).*
2. *To define population-specific transmission profiles between predominant whitefly biotypes and TYLCV in tomato, and other geminiviruses of vegetable crops.*

Specific Objectives

1. *To identify primers that will differentiate between Egyptian populations of Bemisia tabaci, the vector of tomato yellow leaf curl, collected from isolated geographic locales in crop and weed hosts of the whitefly,*
2. *To investigate the degree of relatedness between Egyptian whitefly vector populations and those in the adjacent regions,*
3. *To corroborate biological (host preferences, vector relations), biochemical, and molecular data sets for populations studied in the AZ and AGERI laboratories.*

Major Accomplishments

- Documented the distribution of distinct whitefly vector populations in Egypt using biochemical and two molecular markers in collaboration with AGERI scientists.
- Initiated tracking of distinct vector populations and geminiviruses associated with vegetable crops, particularly tomato, throughout Egypt and within the region.
- Initiated efforts to develop a computer based approach for rapid identification and tracking of whitefly biotypes as a management tool accessible via the World Wide Web.
- Established whitefly vector colonies at AGERI and to conduct whitefly-transmission experiments with tomato yellow leaf curl virus using two whitefly species, *B. tabaci* and *T*. *ricini*. Substantial work was conducted with *T. ricini*.

Other Activities

- Scientific communications between laboratories by email and fax.
- Coordinate ID of whiteflies with R. Gill (California State Dept. of Agriculture)
- Several visits by AGERI scientists to Arizona laboratory to facilitate the collaboration, accomplish whitefly identification with the expert assistance of Mr. Rafael Caballero (pH student), and to complete initial PCR and cloning of 1997-98 whitefly collections with Ms. I. Torres-Jerez (Research Specialist). The AZ PI visited Egypt several times during the project to discuss results and make plans to execute project objectives in subsequent stages of the project. In May, 1998 the UA PI visited Egyptian scientists with the project team to finalize the project and complete final reports.

Goals not accomplished

Whitefly vector-mediated transmission experiments were not pursued in as great of depth with *B. tabaci* as was anticipated. It was difficult to establish working whitefly colonies and difficult to conduct these tedious experiments at AGERI. We had hoped to be able to equate differences or similarities in vector mediated transmission of several candidate geminivirus isolates with molecular sequence typing of *B. tabaci* vector biotypes

Description of Activities and Methods of Work

Molecular markers for whiteflies

To initiate this project, prototype reference populations from globally representative biogeographies world were first examined to determine the extent of variation within and between several distinct *B. tabaci* populations (Frohlich et al., in preparation). We examined two molecular markers for reference whitefly populations in advance of examination of the more precious collections from Egypt and adjacent geographic areas. Initially, the regions 3-5 of the 16S ribosomal RNA gene and the 5' end 550-600 bases of the mitochondrial gene COI were examined. The utility of these mitochondrial genome regions for inferring whitefly relationships and their for application as a diagnostic sequence to achieve identification of whiteflies within the *B. tabaci* species complex varied with the marker examined.

Clustal analysis or pairwise distance measures, parsimony sampling of aligned sequences to determine the shortest possible phylogenetic tree (fewest genetic changes), and frequency analysis using EXCELL spreadsheet method (from Dr. Claude Fauquet's phylogenetic work with plant geminiviruses) were used to assess the utility of the two prospective molecular markers. Results indicated that Clustal analysis of either 16S ribosomal gene fragment or of the COI fragment support phylogenetic trees that readily separated whiteflies into clusters having a strong geographic basis, relative to the collection origin of the sample. The only exception was the B biotype which was proposed to have an origin in the Eastern Hemisphere origin, but has become established in many world regions. These data confirmed the proposed Old World origin of the B biotype which routinely clustered with other Old World *B. tabaci* populations. In addition, Excell spreadsheet frequency analysis of select distance data for 16S and COI sets indicate COI sequences may separate geographically adjacent populations in discernible genotypes more effectively than 16S sequences.

Although both molecular markers permitted placement of the population in the geography of its origin (Eastern or Western Hemispheres), the COI marker sequence exhibited greater variation than did 16S sequences. To validate the efficacy of both molecular markers, it was necessary to examine 16S and COI sequences for a well-characterized subset of whitefly populations. Data were evaluated by several accepted methods of sequence analyses to validate or dispute the prospective molecular markers for distinguishing whiteflies at the genus, species, and subspecies levels. Results indicated that the COI gene sequence was more informative than the 16S (Frohlich et al., in preparation) and was subsequently selected as the more optimal marker for all future studies. In conjunction with a sister project ongoing in the AZ laboratory, we were able to extend this analysis to *B. tabaci* populations from the Americas, and the Caribbean Basin to establish the extent of genetic variation that occurs between whitefly vector populations in Egypt and more distant geographic sites. Several collections of whiteflies from some countries adjacent to Egypt are available in the AZ laboratory; however, we have a substantial collection from Southeast Asia, North and Central America, and some representatives from South America that will constitute a selection of populations from which to obtain additional COI reference sequences. For this project, we recently completed cloning and sequencing the COI marker from multiple whitefly samples of *B. tabaci* and *T. ricini* from Egypt in 1997-98. Editing of sequences is underway to obtain a single consensus sequence per sample.

Ultimately all edited COI sequences will be complied and placed in an interactive comparative DNA sequence data base that will be accessible on the World Wide Web. Users may obtain a rapid identification of the whitefly in question by PCR-amplifying the target region of the COI marker gene, obtaining the DNA sequence, and comparing it interactively to reference COI sequences available at the site. For the most part, biological studies are lacking for *B. tabaci* populations that can be corroborated with their identity and distribution. Once populations are mapped, we will begin to collect and collate available biological information for specific populations as they are studied by local researchers.

It should be pointed out that this is the first effort of its kind to facilitate accurate identification of *B. tabaci* , a requisite to rationale implementation of strategies toward control of whiteflies as pests and vectors of geminivirus-incited diseases. This project involved an initial effort to define informative genotype-specific DNA markers for identification of populations of *B. tabaci* using those from Egypt as geographic reference populations for the region. Upon completion of consensus sequences and phylogenetic analysis, we will have succeeded in our initial effort to map the identity and the extent of variation of *B. tabaci* in the most important vegetable and fiber crops in Egypt. Analyses of the Egyptian and AZ laboratory reference subset sequences will be completed before the end of 1998. We expect the website database will be under construction by early 1999.

PCR primers for detection/identification of geminiviruses in the whitefly vector

We evaluated the utility of general primers (Wyatt and Brown, 1996) that facilitate the detection of whitefly-transmitted geminivirus DNA in field-collected whiteflies that were either dried or stored in 70% ethanol. This capability is seen to be key to linking vector potential to a particular whitefly biotype population that is found in association with a particular crop and weed species in Egypt. Further, geminiviruses in Egyptian crops remain largely unstudied. We applied this approach to identifying geminiviruses in field samples based upon the ability of the molecular core Cp marker to establish a tentative virus identification by its comparison with reference virus sequences (Brown et al., submitted). This work was accomplished in the AGERI laboratory using primers developed and supplied by the AZ laboratory. AGERI also utilized primers of their own design and compared the results obtained using numerous virus-specific and broad PCR approaches. In all cases, geminiviral PCR products were cloned and the sequences obtained to determine virus identity. of Identification in which the core Cp primers were implemented was achieved by matching candidate sequences with reference sequences of well-studied geminiviruses available in the Arizona database. Virus sequences obtained thus far will serve as signature sequences for future work in determining the distribution of geminiviruses in crop and weed species in Egypt.

Technology transfer and scientific exchange

Our Egyptian cooperators visited the Arizona laboratory several times during the project, with the most recent visit in February, 1998. During which time, we initiated work on analysis of the first collection of whiteflies from Egyptian crop and weed species. The work accomplished involved (a) morphologically-based taxonomic identification of 28 whitefly samples, (b) initiation of molecular analysis (PCR), (c) demonstration and trial run to molecularly clone several COI fragments obtained by PCR amplification of the marker gene. In addition, we provided PCR primers, molecular biology supplies, and protocols from our laboratory to enable continuation of the work with additional samples upon the return of our collaborators to Egypt. Results indicated that whiteflies from seven castor bean samples were *T. ricini*, the castor bean whitefly, while the castor bean whitefly and the geminivirus vector, *B. tabaci*, were found to be mixed on cabbage and tomato. The remainder of the multiple samples from cabbage, cotton, cucumber, eggplant, lettuce, okra, pepper, and squash were identified as *B. tabaci*.. *B. tabaci* populations were extracted and used for PCR of the mitochondrial COI fragment. When final sequences are obtained, a phylogenetic tree will be generated that infers relationships between populations from Egypt and reference populations from representative world sites. In addition, all sequences will be placed in GenBank, with Egyptian sequences submitted jointly by the US-AZ and AGERI laboratories. A map will be compiled at the AZ-based Whitefly Link website that illustrates the worldwide distribution of COI variants in relation to *B. tabaci* biotypes and their association with geminivirus distribution. A manuscript for publication in a professional journal will be co-authored by the two laboratories that will describe the variation in *B. tabaci* from Egypt based upon COI gene sequences.

We also provided core Cp primers and protocols established in the Arizona laboratory (Wyatt and Brown, 1996; Brown *et al*., in preparation) for detection of geminivirus DNA in single whiteflies and plants. These primers were used in preliminary experiments conducted at AGERI.

Future Work

Farmers presently have no way of assessing the identity of whitefly populations, some of which are damaging and should be controlled with chemical pesticides, where as others are relatively harmless. Discriminative pesticide use coupled with biological control approaches will lead to more sustainable management of pest and vector problems associated with whitefly infestations in Egyptian crops. Accurate identification of whitefly species and subspecies will now be possible using molecular methods developed and tested jointly by AZ and AGERI scientists. As additional reference sequences are added to the data base, identification of whiteflies from other regions will also be possible. Other important applications for COI molecular diagnostic system will be the ability to detect introduced pests immediately upon introduction or dispersal, and likewise, detection of new indigenous populations that have not been problematic in the past.

Further, the ability to identify and track geminiviruses in plants and in vector populations will facilitate selection of the most important and virulent viruses against which resistance programs should be directed. We have learned that many whitefly-transmitted geminiviruses exist in Egyptian crops, but the majority remain unidentified. Likewise, the level of damage they inflict is not known, nor are the biotic properties (host range, virus-vector interactions) or molecular characteristics documented. Geminiviruses of greatest economic priority should be molecularly cloned to facilitate implementation of genetically engineered crop protection approaches involving virus-derived resistance and traditional efforts to develop disease resistance through plant breeding. This work provides the first tool for accurately assessing a large number of plant or whitefly samples to determine the identity and distribution of viruses that should be targeted in disease control programs. In conjunction with whitefly biotype identity, it will be possible for the first time to know 'who and where the enemy is, the first step toward effective disease and vector control. Future collaborative efforts are envisioned in which AZ and AGERI cooperate to obtain additional reference samples for which COI sequences will be obtained and entered into the global data base. AGERI will make collections in the Mediterranean, Middle and Far East regions, while AZ will cover the Americas, Southeast Asia, and the Indian subcontinent. As mentioned, some collections are already in place in the AZ laboratory.

Future work is expected to utilize these tools and technologies now in place at AGERI. Additional optimization under AGERI conditions and analysis of additional COI whitefly and core Cp virus sequences for new whitefly collections is highly feasible and will promote continued collaboration as well as a capacity on the part of AGERI to independently carry out whitefly and geminivirus identification. We expect that a regional service lab located at AGERI could be a reality in a fairly short period of time, given AGERI can charge a nominal fee for identification services to farmers and consultants in Egypt and throughout the region. Establishing a full capacity to accurately identify virus and whitefly samples will be enhanced by the addition of new reference whitefly COI and virus core Cp sequences to established virus and COI data bases established in AZ but accessible to scientists at AGERI through the World Wide Web.

Additional future work envisioned in the AGERI laboratory, in part, involves selecting the most important previously unstudied geminiviruses for detailed molecular and biotic characterization, in cooperation with the Arizona laboratory. Tomato yellow leaf curl has been the focus of much resistance work at AGERI, but resistance to other geminiviruses affecting other crops is needed. Also, continued application of PCR primers for virus detection will eventually permit the construction of a map illustrating the distribution of geminiviruses and their whitefly vector biotypes in Egypt and in adjacent sites that may impact Egyptian agriculture. Ultimately, a geminivirus and whitefly data base will be accessible on the World Wide Web. This construction project is underway in the Arizona laboratory, but can already be utilized for interactive comparative identification of geminiviruses.

Potato Transformation for Development of Potato Tuber Moth Resistance

Lead Principal Investigators

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

Project Partners

Taymour El-Nasr and Magdy Makour, AGERI, Egypt

Potato Project Objectives

- Genetically engineer potato varieties important to Egypt that will control potato tuber moth with emphasis on genes available for commercial development.
- Examine the foliar expression levels of transformed potato lines for potato tuber moth control in laboratory (MSU and AGERI) and field tests in Egypt.
- Evaluate the effectiveness of tubers stored in nawallas (ambient temperature storage structures) in controlling potato tuber moth.
- Evaluate the effectiveness of individual and combined resistance factors under laboratory experiments.
- Train scientists from Egypt in techniques of genetically engineering the cultivated potato, conduct field trials under biosafety guidelines, and evaluate insect resistance under field and laboratory environments.
- 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 Problem

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

Potato tuber moth, *Phthorimaea operculella* Zeller, is the most serious insect pest of potatoes in sub- and tropical latitudes. Insecticide use is the most common means of potato tuber moth control in both field and storage. Twelve to twenty insecticide applications are commonly used to control potato tuber moth during the growing season, three to four insecticide sprays or dusts are often applied for potato tuber moth control in storage with the last application within 1 week of marketing (Madkour 1999).

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

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

Potato Project Research

Initially, transformations with the *cryla(c)* wild type gene were performed using cv. 'FL1607' as a model system (Hudy et al., 1995). 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 *Bt-cry5* construct (with the *gus* gene fused to the *Bt-cry5* gene) was used in transformations with cvs. 'Lemhi Russet', 'Atlantic', L235-4 (glandular trichome line), and USDA8380-1 (foliar leptine line) (Westedt et al. 1998). The *Bt-cry5* constructs that differ in the promoter (CaMV 35S, Gelvin super promoter and patatin promoter) were transformed into cv. 'Spunta' (Li et al. 1999). The *Bt-cry5-PVYcp* gene construct was also transformed into 'Spunta' (Li et al. 1999). Spunta is the most important cultivar grown in Egypt and is used for local consumption, while Atlantic is a desired chip-processing cultivar. Other constructs that have the *gus* gene removed are ready to use in transformation. A sample of the *Bt-cryl* and *Bt-cry5* transgenic lines was transferred to AGERI as tissue culture plantlets for greenhouse testing.

Detached leaf bioassays are used to determine the level of host plant resistance to potato tuber moth. Various potato lines were screened for natural resistance to potato tuber moth. All PCR-positive Bt-transgenic lines developed from this project were screened for resistance to potato tuber moth. In addition, a series of other transgenes were evaluated but had no effect upon potato tuber moth mortality. We also obtained a number of synthetic *Bt-cryla*-transgenic potato lines from the USDA to test; these lines gave strong control of the tuber moth. The most promising lines from the detached leaf tests were also advanced to laboratory tuber bioassays. Tuber bioassays identified a series of *Bt-cry5*-Spunta and *Bt-cry5/PVY*-Spunta with high levels of potato tuber moth mortality (Li et al 1999). Other *Bt-cry5*-transgenic lines (Atlantic, Lemhi Russet and L235-4) were less effective in controlling the tuber moth, but were significantly different from the non-transgenic cultivars.

Agronomic evaluation of the Bt-transgenic potato lines was initiated in Michigan in 1994. Yearly agronomic evaluations have been conducted at this location and the trial size has varied 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 (Douches, et al. 2002b). 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 each year (1996 – present).

The first field test of genetically engineered potatoes in Egypt occurred in January 1997 at AGERI after the Egyptian biosafety regulations were established. The purpose of this trial was to evaluate an array of Bt-transgenic potato lines for field resistance to potato tuber moth. Fourteen lines were evaluated for foliar and tuber damage. To apply greater tuber moth pressure, the field was artificially inoculated during the season. Foliar mining was as high as 38 mines per 10 untreated plants, whereas the Bt-lines had as few as low as 1 mine per 10 plants. Non-transgenic tuber infestation was 80-92% (severe level of infection). In contrast, some of the Bt-transgenic lines had as little as 38% infection of the tubers. These results were very promising and expanded field trials were established for 1998 in Egypt. In February, AGERI trial was repeated and an insect and an agronomic trial were planted at the CIP Potato Research Station (located in the delta potato-producing region).

A 1999 field trial was conducted in Egypt at AGERI and CIP-Egypt. This trial identified lines with excellent control of potato tuber moth including Spunta-G2 and Spunta-G3 that had virtually no potato tuber moth infestation compared to the non-transformed control lines in our CIP field trial (Douches et al., 2002a). Storage experiments were conducted with the harvested potatoes and again the Spunta-G2 and Spunta-G3 lines had minimal infestation for nearly 3 months in storage.

A 2000 field trial was conducted in Egypt at AGERI and CIP-Egypt. The results again demonstrated excellent control of potato tuber moth from several lines with Spunta-G2 and Spunta-G3 having minimal numbers of mines in the foliage and tubers at harvest. Storage experiments were conducted with the harvested potatoes and results show that the transgenic 'Spunta' lines had very few infested tubers compared to the non-transformed controls.

New constructs using four different for *Bt-cry5* expression were developed. The different promoters include CAMV 35S, Gelvin super promoter (GSP), Patatin, and Ubiquitin3. The Ubiquitin3 promoter was developed by USDA and thus could eliminate several IPR restraints. This promoter is being used to design a freedom-to-operate vector, which may hasten commercialization of our transformed potato lines.

'Atlantic', 'Lady Rosetta' and Jacqueline Lee (MSU late blight resistant line) transformations with a *Bt-cry5* vector (pSPUD5) have been completed. Atlantic and Lady Rosetta are important chip varieties in Egypt and are key for Egyptian commercialization. Jacqueline would offer a combination of late blight resistance (major production constraint) and potato tuber moth resistance.

A 2001 field trial was conducted in Egypt at CIP-Egypt. The results show excellent control of PTM from our transgenic lines. Storage experiments were conducted with the harvested potatoes and the results show that Spunta-G2 and Spunta-G3 provided excellent control of the potato tuber moth (Douches, et al., 2002b). In addition, ecological data was collected in the region relating to potato tuber moth and other important insects found in the potato producing region.

Seed production increases for year 2001 field trials in Egypt were made at the MSU Montcalm Potato Research Farm, Montcalm Co., MI. Additionally, greenhouse tubers were produced by the private sector for field trials yielding 2000 seed pieces of various 'Spunta' lines (Spunta G2, Spunta G3, Spunta S1, Spunta S4 and Spunta 6a-3 [*PVYcp/Cry5*]). Our plans were to test these lines in Egypt on commercial farms in 2002. Planting these lines on commercial farms would have allowed growers to see the benefits of these lines and hasten commercialization. However, due to Egyptian plant registration laws we were

not allowed to test on commercial farms. Agronomic trials were conducted in Michigan testing the *Bt-cry5* Spunta lines. All lines were comparable to the non-transformed control.

Greenhouse plantlets of transgenic Atlantic, Lady Rosetta and Spunta lines were produced for leaf bioassays. Detached-leaf feeding bioassays were conducted in the lab with 'Lady Rosetta' and 'Atlantic' lines transformed with the different constructs. Results of these tests indicate that most of the transformed plantlets provide excellent control of potato tuber moth. Molecular analysis was conducted on these lines to verify the number of genes inserted and amount of protein expressed.

A 2002 field trial was conducted in Egypt at CIP-Egypt. The results show excellent control of PTM from our Spunta-G2 and Spunta-G3 transgenic lines. However, due to low numbers of potato tuber moth the nontransformed Spunta lines also had little damage. Storage experiments are underway with the harvested potatoes and we are waiting for the results of these tests. Limited ecological data was collected because of travel constraints in Egypt.

An effort was initiated to develop a process for a humanitarian release of the Bt-Spunta potatoes in Egypt and South Africa. This effort involves MSU/ABSP, USAID, Syngenta, CIP, AGERI/Egypt and VOPI/South Africa.

Lessons Learned

- Communication important; cultural differences can limit or impede communication.
- Training of developing country scientists valuable; helps in establishing communication; long-term training much more valuable and effective than short-term training.
- Trips to target countries stimulate research progress.
- Vector construction is a continuous process, not a final step.
- IPR issues are a changing target.
- Biosafety and IPR issues are as important as research when commercialization is the final goal.
- Transgenic plants must be in hand to establish regulations, food safety testing and field testing.
- Need to address social, economic and ethical issues in research.
- Food safety issue larger than expected.
- Anti-GMO issues developed and limited progress towards commercialization. GMO plants became more regulated rather than less regulated.
- GMOs became a trade barrier during the project and limited commercialization efforts.
- Need to have and maintain a complete linkage between lab and field research to make progress towards commercialization.

Project Accomplishments

- Engineered vector constructs and expressed *Bt-cry5* gene in over 200 potato lines.
- Over 40 different Bt-lines were field tested in Michigan and/or Egypt.
- Conducted studies to evaluate the effect of combining natural resistance mechanisms with *Bt-cry5.*
- First field trial of transgenic plants in Egypt in 1997. This field trial was the main incentive for

establishing biosafety guidelines for Egypt.

- Development of commercially acceptable Bt-Spunta lines to control potato tuber moth in the field and storage.
- Evaluated the efficacy of the *Bt-cry5* gene expression against other potato insect pests.
- Initiated food safety assessment of Bt-Spunta lines in collaboration with Germany/AGERI.
- Trained scientists from Egypt in techniques of genetically engineering the cultivated potato, conducting field trials under biosafety guidelines, and evaluating insect resistance under field and laboratory environments.

Publications

Coombs, J.J., D. S. Douches, W. Li, E.J. Grafius, and W.L. Pett. 2002. Field evaluation of natural, engineered, and combined resistance mechanisms in potato (*Solanum tuberosum* L.) for control of Colorado potato beetle (*Leptinotarsa decemlineata* Say). J. Amer. Soc. Hort. Sci. (in review).

Coombs, J.J., D.S. Douches, W. Li, E.J. Grafius, and W.L. Pett. 2002. Combining engineered (*Bt-cry3A*) and natural resistance mechanisms in potato (*Solanum tuberosum* L.) for control of the Colorado potato beetle. J. Amer. Soc. Hort. Sci. 127:62-68.

Douches, D.S. and E.J. Grafius. 2002. Transformation for insect resistance. In: Genetic Improvement of Solanaceous Crops, Vol.2: Potato (Eds: M.K.Razdan and Autar K. Mattoo), Science Publishers Inc., Enfield, USA. (submitted)

Douches, D. S. and W. L. Pett. 1995. Potential environmental concerns *vis-a-vis* the introduction of a specific trait: Experience with potatoes engineered to express insect or virus resistance. In: Environmental concerns with transgenic plants in centers of diversity: Potato as a model. (eds.) R. J. Frederick, I. Virgin and E. Lindarte. The Biotechnology Advisory Commission, Stockholm Environment Institute and Inter-American Institute for Cooperation on Agriculture. Pp. 45-52.

Douches, D.S., A. Westedt, Liswidowati, A. Hadi-Permadi, W. Pett, and E. Grafius. 1996. Progess in development and evaluation of Bt-transgenic potatoes with resistance to potato tuber moth (*Phthorimaea operculella*). Am. Potato J. Vol. 73: 524.

Douches, D.S., A.L. Westedt, K. Zarka, and E.J. Grafius. 1998. Transformation of *CryV-Bt* transgene combined with natural resistance mechanisms for resistance to tuber moth in potato (*Solanum tuberosum* L.) HortScience 33(6):1053-1056.

Douches, D.S., E. Grafius, W. Pett, K. Maredia, J. Brink, M. Madkour, T. Nasr-El Din, C. Ives. 2002. A Case Study of Using Biotechnology for Pest Management in Potato in Developing Countries. (In preparation)

Douches, D.S., W. Li, K. Zarka, J. Coombs, W. Pett, E. Grafius and T. El-Nasr. 2002. Development of *Bt-cry5* Insect Resistant Potato Lines Spunta-G2 and Spunta-G3. HortSci. (in press).

Douches, D.S., W. Pett, F. Santos, J. Coombs, E. Grafius, W. Li, E. A. Metry , T. Nasr El-Din and M. Madkour. 2002b. Field and Storage Testing *Bt*-Potatoes for Resistance to Potato Tuber Moth (Lepidoptera: Gelichiidae). J. Econ. Entomol. (in review).

Douches, D.S.,T.J. Kisha, W. Li, W.L. Pett, and E.J. Grafius. 2001. Effectiveness of natural and engineered host plant resistance in potato to the Colorado potato beetle (*Leptinotarsa decemlineata* (Say)). HortSci. 36:967-970.

Hudy, P., D. Douches, W. Pett, and E. Grafius. 1995. Evaluation of natural resistance, transgenic resistance and the two combined, for control of potato tuber moth (Phthorimaea operculella). Am. Potato J. 72(10): 639.

Li, W., K. Zarka, D.S. Douches, J.J. Coombs, W.L. Pett, and E.J. Grafius. 1999. Co-expression of potato PVY^0 coat protein gene and cryV-Bt genes in potato (*Solanum tuberosum* L.). J. Amer. Soc. Hort. Sci. 124(3):218-223.

Mohammed, A. D.S. Douches, W. Pett, E. Grafius, J. Coombs, Liswidowati, W. Li, and M.A. Madkour. 2000. Evaluation of potato tuber moth (Lepidoptera: Gelechiidae) resistance in tubers of *Bt-cry5* transgenic potato lines. J. Econ. Entomology 93(2):472-476.

Pett, W. L., D. S. Douches, and E. J. Grafius. 1997. Development and field testing of transgenic potatoes with putative resistance to potato tuber moth. In: Japan-U.S.A. Common Agenda. Seminar on seed potato multiplication in Indonesia. Seminar Secretariat: Seed Potato Multiplication and Training Project. Japan International Cooperation Agency. pp1-7.

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.

Westedt, A.L., D.S. Douches, W. Pett, and E.J. Grafius. 1998. Evaluation of natural and engineered resistance mechanisms in *Solanum tuberosum* for resistance to *Phthorimaea operculella* (Lepidoptera: Gelechiidae). J. Econ. Entomol. 91 (2): 552– 556.

Literature Cited

Georgiou, G. P. and C. E. Taylor. 1986. Factors influencing the evolution of resistance. In Pesticide Resistance: Strategies and Tactics for Management. National Academy Press, Washington, DC.

Gould, F. 1986. Simulation models for predicting durability of insect-resistant germ plasm: a deterministic diploid, two-locus model. Env. Ent. 15(1): 1-10.

Madkour, M. 1999. Addressing agricultural development in Egypt through genetic engineering. Advances in Agricultural Research in Egypt. 2(2):115-135.

McGaughey, W.H. and M.E. Whalon. 1992. Managing insect resistance to Bacillus thuringiensis toxins, Science 285:1451-1454.

Perlak, R.J., R.L. Fuchs, D.A. Dean, S.L. McPherson, and D.A. Fischhoff. 1991. Modification of the coding sequence enhances plant expression of insect control protein genes. Proc. Natl. Acad. Sci. 88:3324-3328.

Tabashnik, B.E. 1994. Evolution of resistance to Bacillus thuringiensis. Ann. Rev. Entomol. 39:47-79.

Yadav, N.R. and M.B. Sticklen. 1995. Direct and efficient plant regeneration from leaf explants of Solanum tuberosum L. cv. Bintje. Plant Cell Reports 14:645-647.

Equipment Purchased

An upright -80°C Freezer for storage of DNA, biochemicals and bacterial cultures used in genetic engineering and vector construction. It was purchased in 2000 for approximately $8,000. It is located in 499A Plant and Soil Sciences Building. This unit will be used for continued storage of above-mentioned materials for related research work.

Transgenic Virus Resistant Tomato Project

US Research Team

Dr. Beachy, ILTAB, US coordinator

Dr. Fauquet, ILTAB, Coordination of the project

Dr. Padidam, ILTAB, Molecular biology/Virology

Dr. A. De Gonzales de Schopke, ILTAB, Cell Biology/Transformation

Egyptian Research Team

Dr. M. Madkour, Egyptian coordinator

Dr. Abdallah, AGERI, Molecular biology

Dr. Aref, AGERI (until 96), Molecular Biology/Virology

Dr. El-Bakry, AGERI(until July 94), Cell Biology/Transformation

Mohamed El-Wahed, Cell Biology/Transformation

Objectives of the Project

The practical goal of the project is the production of transgenic tomatoes resistant to the tomato yellow leaf curl virus disease from Egypt (TYLCV-Eg).

Tomato yellow leaf curl disease is a very devastating disease, all over the tropical countries in Africa, Middle East and South-East Asia. The severity of the disease is depending on the epidemiology of the whitefly vector, but as whiteflies are invading new ecological territories, TYLCV becomes a threat in producing areas. And there are virtually no efficient control method to prevent the development of the disease: there are no suitable resistant varieties available, no agricultural techniques easy to use, and no treatments against the vector nor against the virus when the plant is infected. Tomato yellow leaf curl disease has been reported in many tropical countries in the world, especially in West Africa, in East Africa, in the Middle East, in India and in Thailand. The disease is also now invading temperate zones and it has been recently reported in Sardinia, Sicily and Spain. The losses can be extensive and can eliminate production entirely. The disease currently prevents tomato culture in several West African countries during the winter season and cause high losses during the summer season. In Egypt, of the production of 484,963 ha., the losses have been estimated to a range of 5 to 35% depending on the season of production. Current and past agricultural practices are either ineffective or are only partially efficient, and insecticide treatments are unable to control the vector.

To reach the goal the US-Egyptian research team has to study the virus causing the disease at the molecular level, to establish a molecular strategy to control the virus and to transfer the viral genes, involved in this strategy, into Egyptian tomato varieties. These tomatoes will have then to be challenged in good conditions and if positive they would have to be evaluated by breeders for their production or they would have to be used as parental lines for transferring the gene of interest into the appropriate genotype.

Summary of Research Achievements

The stated goals of the 'Tomato-Trans Project were:

- To establish a collaborative research project with Egyptian counterparts at AGERI, Cairo, Egypt, for exchange of information, reagents, and technology relative to the diagnosis and control of geminivirus diseases in tomato for Egypt;
- To develop strategies and reagents for the diagnosis of such viruses for use in Egypt and other relevant regions of the world;
- To develop strategies via plant genetic transformation to develop transgenic plants that are resistant to tomato yellow leaf curl virus (TYLCV) a major disease of tomatoes in Egypt.

During the course of the Trans-Tomato Project we have made significant progress in each of the targeted areas, as summarized here:

- Several clones of TYLCV-Eg have been obtained by AGERI scientists. The sequence of the tomato clone has been done. The TYLCV-Eg isolate is a strain of the virus described in Israel and is a different virus from Italy and Spain.
- Using computer-assisted comparisons of the geminivirus database we developed oligonucleotide primers that can be used to identify whitefly-transmitted geminiviruses. The primers have been tested at ILTAB and at AGERI, and have been distributed to other colleagues in the world for field diagnosis of whitefly-transmitted geminiviruses. Many geminivirus isolates have been cloned and characterized molecularly and biologically.
- A large number of chimeric genes have been constructed using sequences derived from the genome of TYLCV-Eg. Each of the constructs were developed with the enhanced 35S promoter of cauliflower mosaic virus, and have been used in tomato transformation experiments. All of the constructs have been provided to AGERI.
- We have produced polyclonal antibodies against the coat protein, the pre-coat, and the replicase of TYLCV-Eg. These antibodies which have a high specificity and good titles, have been made available to AGERI scientists. The antibodies for the coat protein are also capable of detecting any whitefly transmitted geminivirus, as they were designed expressly for that purpose in order to be used for other geminiviruses.
- A new strategy of pathogen derived resistance for control of geminiviruses using the 'pre-coat' gene of geminiviruses has been undertaken. Transient assays that were carried out gave indication that certain mutant proteins can act as dominant negative, inhibitors of disease development. A gene encoding such a dominant negative protein has been integrated into *N. benthamiana* and tomato plants: the transgenic tobacco plants have been characterized and tested for resistance, while the tomatoes are being characterized. The tobacco plants were not expressing the pre-coat protein showing a particular problem associated with this protein and therefore there was no resistant phenotype. The tomato plants will be tested as it is possible that the plant host is important for the development of such strategy.
- Another strategy of pathogen derived resistance for control of geminiviruses using non geminiviral proteins has been tested. Expression with a geminivirus vector of that protein in protoplasts is leading to a drastic modification of the virus replication. When co-bombarded with a wild type geminivirus this protein prevents the virus to move systemically and the plants does not show symptoms. Transgenic tobacco and tomato plants have been made and study of the expression of this protein with a variety of constructs to increase our chances of a good level of expression for getting a resistant phenotype is undergoing. If successful this strategy could lead to a universal control of geminiviruses, in any plant.

- Tomato transformation has been firmly established with both marker genes and genes derived from the genome of TYLCV-Eg: to date, more than 240 transgenic lines have been developed. The protocol adopted has led to frequencies of transformation approaching 9% in selected experiments. Transfer of the successful protocol to AGERI has been achieved and tomato transformation can be carried out in Egypt.
- The transgenic tomato lines have been challenged at the F1 and subsequent generations, and segregation of the transgene has been evaluated. As most of the lines do not express any resistance it appears that tomato plasg the Rep coding sequence in antisense (AC1 gene) are capable of limiting the virus infection and therefore showing resistance to the TYLCV. This type of results has also been obtained for other geminiviruses as well. However, the tomato plants engineered are a Californian type of tomato used as a model tomato plants. This strategy will have to be transferred into Egyptian grown tomatoes. The results of the challenging tests will be provided by the Egyptian team.

Research Activities

Characterizing geminiviruses that cause diseases in tomato in Egypt.

A significant component of the research project is to document the extent of the geminivirus related diseases in Egypt. It is obvious that there are symptoms of yellow leaf curl on tomatoes in many areas of Egypt, but there are other virus-induced symptoms as well. It is not yet clear whether or not the sole pathogen of concern is TYLCV-Eg, and whether or not other strains of geminiviruses infect tomatoes in Egypt. To determine the extent of the variability of geminiviruses (and, possibly, other virus diseases) and to select and/or develop the strategies that will be most successful we have:

- Developed a database with convenient criteria to allow one to determine if a particular virus is a strain of a previously characterized virus, or is a new virus;
- Isolate geminiviruses from various areas in Egypt that cause different disease symptoms;
- Evaluate the new virus isolates in molecular and biological characteristics;
- Determine whether or not the isolates can cause disease symptoms and yield losses comparable to those observed in the field, either alone, or in combinations.

Item (1) has been completed by the US team and led to the production of a diagnostic kit for geminiviruses and publication of a manuscript. Items (2) and (3) have been initiated by the Egyptian group and a lot of clones have been accumulated. The conclusions of the study are that geminiviruses are fairly homogenous in their nucleotide sequences and those sequences most informative for comparative purposes are at the 5' end of the coat protein sequence. This finding allowed up to design specific primers to amplify sequences from essentially any whitefly transmitted geminivirus for subsequent sequence analysis. Such analyses will determine if the amplified DNA is a strain of a previously described virus or an undescribed virus. Primers have been provided to the Egyptian team for their use with cloned DNAs and collected plant samples. Primers have also been supplied to other research groups in the world, like in Mexico, Arizona and India. After gathering the information obtained by these groups this kit cold be made available on a large scale.

Gene constructs containing TYLCV-Eg sequences.

A variety of chimeric gene constructions have been made with sequences from TYLCV-Eg, including:

- CP (AV1) sequences in the sense and antisense orientations;
- Precoat (AV2) and CP (AV1) sequences in the sense and antisense orientations;

- Precoat (AV2) sequences in the sense and antisense orientations, mutated or not;
- Replicase (AC1) in sense and antisense orientations;
- Replicase (AC1) in which the NTP binding site is mutated.

In each case the gene sequence was placed under control of the CaMV 35S promoter in an intermediate plasmid, and transferred to *Agrobacterium tumefaciens for* plant transformation. The AC1 mutated strategy has been proved to be capable of producing resistant transgenic tobacco plants with another geminivirus and therefore there are good reasons to believe that it will work for TYLCV as well, although this remains to be shown in tomatoes. The plants have been produced and will be transferred soon to Egypt for challenging.

Production of TYLCV specific antibodies and geminivirus generic antibodies.

ILTAB previously agreed to develop and test antibodies against the pre-coat, coat protein, and replicase genes using proteins expressed in *E. coli* and specific oligopeptides. Rabbits have been injected with expressed pre-coat and coat proteins, and serological tests demonstrated specific antibody-antigen reactions in western blots. We never been able to express the replicase in E. coli therefore no antibodies were produced. Antibodies have also been produced with oligopeptides for the coat protein and the replicase, the first ones are available and working in both western and Elisa. On the contrary the antibodies raised against the replicase only work in Elisa and not in western.

Transformation of tomatoes.

Since the first year of the project we had excellent results in our tomato transformation experiments and we produced a total of 267 transgenic tomato lines. Some of these plants contained the TYLCV-derived gene of interest and have produced seeds. The first seeds available have been transmitted to Egypt after preliminary characterization completed at ILTAB. Improvements in the handling of explants, improved transformation protocols, and experience led to more routine transformation of UC82B, and transfer of relevant technologies to Egypt.

Transformation experiments initiated at ILTAB with Dr. El-Bakry and M. El-Wahed in December 1993 were repeated and/or completed with tomato cultivars that are currently in use in Egypt. These studies confirmed that transformation carried out in the presence 20 mM proline somewhat improved the efficiency of transformation (from 1.5% of explants to 3%, in several experiments). Another set of experiments using sorbitol as a carbon source showed a very high increase in transformation efficiency (6% vs 1.3% with sucrose), despite the fact that the percentage of callus formation was lower with sorbitol than with sucrose. In view of these results, additional studies are being carried out in order to come up with a publishable report in association with AGERI.

Production, characterization and challenge of transgenic tomato lines expressing TYLCV genes.

About 200 lines of transgenic tomatoes have been produced and are being characterized for the presence and for the level of expression of the foreign genes, this work is still underway and should be finished by the end of the current project. The characterization has been done by PCR at the first generation and by southern, and northern blots at the second generation. However, all lines, and particularly those containing the antisense replicase constructs, have been transferred to AGERI for challenging. The plants were submitted to whitefly inoculation by putting the healthy transgenic lines in close contact with infected tomatoes covered with whiteflies. The results indicated that most of the lines were infected but some others (AC1 antisense construct) showed delay of infection. Seeds have been collected from those lines and the subsequent generations of plants have been checked for segregation and resistance. Obviously the molecular characterization of all the transgenic lines will take time and

even more time will be needed for the challenging. Unfortunately, some technical problems and lack of manpower prevented us to obtain the mutated replicase plants in the first phase of the program and that remains to be done.

Development of a new strategy of control.

A new strategy was recently conceived based upon results of studies with TYLCV-Sar and ToLCV-Au that demonstrated that the precoat sequence (AV2) is involved in symptom development. Mutations in AV2 abolished symptoms, and it was proposed that this gene may be involved in long distance movement of the virus. Constructs with AV2 from TYLCV-Eg, in both sense and antisense orientations, were constructed and transgenic plants harboring such constructs are currently under analysis. To demonstrate the role of this gene in TYLCV has not yet been possible due to the poor level of infectivity of the infectious clone of TYLCV-Eg. Therefore, we have carried out such studies with another geminivirus that infects tomatoes in India, ToLCV-In. A complete study of the function of this protein has been carried by ILTAB in order to better understand the role of the precoat in the virus replication, in the movement of the ssDNA or the particles, in the virus encapsidation and in the relationship between the precoat and the coat proteins. Experiences involving extensive mutations, deletions and modifications have been done with whole plants and protoplasts. All this work has lead to a publication in 1996. Selected mutations in this ORF have demonstrated that AV2 is essential for long-distance spread and accumulation of ssDNA. Transgenic tobacco plants that constitutively express this ORF, with or without appropriate mutations, have been developed and R1 progenies have been tested. The fact that tobacco plants were not expressing at all this protein, that we know is a very stable and abundant in the infected plants, prevented us to investigate further the strategy in tobacco. Instead we planned to repeat the work in tomato with the hope that the gene expression would be different, but the substantial decrease of the grant last year and the non-extension of the grant this year prevented us to do so.

Development of a second new strategy of control of all geminiviruses

Even if we get the above strategy to work in tomatoes, which we hope, it will be a very specific strategy capable of controlling only strains of TYLCV and not any other virus. The above study also led us to work on the DNA/protein interactions of the geminivirus DNA with the pre-coat protein and in the course of this study we have identified a category of viral proteins, not from geminiviruses, that have some of the pre-coat protein properties but not others. Transient experiments expressing one of these proteins in a geminivirus vector demonstrated this fact but also showed interference with virus replication. Infection experiments with this vector and the wild type virus showed a high level of reduction of virus replication and no symptoms at all on any of the infected plants. Naturally these transient experiments have to be confirmed with transgenic plants before we could claim resistance with this new strategy. Transgenic tobacco and tomato plants have been made and the plants are being studied for their expression. Anticipating expression problems due to the fact that this protein is a bacterial protein we have started to re-synthesize this gene in order to make it more adapted to plants and we will re-engineer tomato plants if means are available.

Current Status of Research Collaboration with Egypt

The relations with the Egyptian scientists are excellent and the collaboration is good. At each phase of the project a plan of action has been set-up and everybody is following the plans as the results permit it. Currently a number of protocols, primers, gene constructs, antibodies and transgenic tomato seeds have been transferred to Egypt for utilization in the project.

The US partner is in charge of:

- Making constructs with the pre-coat and the replicase,

- Transforming the model plant UC82B with all the constructs (from US and Egypt),
- Producing the transgenic plants at the R1 generation,
- Setting-up a diagnostic system for geminiviruses,
- Setting-up a new strategy of control with the pre-coat gene,
- Producing antibodies against pre-coat, coat and replicase with diverse techniques.

The Egyptian partner is in charge of:

- Producing an infectious clone of TYLCV-Eg,
- Making constructs with the coat and the AC4,
- Transforming the Egyptian varieties with the antisense replicase constructs
- Challenging the transgenic plants at the R2 generation,
- Evaluating the variability of tomato geminiviruses in Egypt,
- Producing antibodies against virus particles.

A kit of primers to diagnostic geminiviruses has been sent to Egypt.

All the chimeric gene constructs made in the US have been transferred to Egypt.

The protocol of transformation has been transferred to Egypt.

The first transgenic seeds of several tomato lines have been transferred from the US to Egypt.

Training of Egyptian Scientists at ILTAB

Dr. Nagwa Aref came 6 months in 1992 to learn cloning, sequencing and gene construction, and 3 weeks in 1993 at ILTAB to perform a comparison between serological diagnostic and PCR diagnostic on a set of samples.

Dr. Naglaa Abdallah, came 9 months in 1991 - 1992 to learn cloning, sequencing and gene construction and 5 months in 1994 to produce several clones with different viral genes.

Dr. Ahmed El Bakry came to the US for about 6 weeks in 1993 to perform transformation of tomato and DNA manipulation.

Mohamed El-Wahed came at ILTAB for a period of 7 months (ending 3-15-94) to learn tomato regeneration and transformation and DNA manipulation.

Constraints and Problems for Achieving the Goals

Budget: the success of this project will firstly depend on the continuity of the funding. Currently funding has been stopped in Sept. 97. The current project has been very good to set up all the tools to work on the TYLCV, but because we had to obtain everything we spent a lot of time in the process. Now that the expertise has been obtained on each side for a number of tools, we can really exploit them for a better achievement.

Biological constraints: Geminiviruses are difficult to control by biotechnology, all the classical strategies are not working or with some difficulty. geminiviruses are special in many ways, so more research will be needed to accomplish their control by genetic engineering. The preliminary results obtained so far are very unexpected and very promising at that respect but even they are confirmed in transgenics before the end of the current project, much more research development will be necessary to transfer them to the field.

Challenge of the transgenics: This part of the project is very crucial and very difficult to achieve, we would have appreciated to have some more input in a better controlled protocol. This would require to involve in Egypt a team of specialists to execute the challenge and possibly a breeder to take in account the plant side.

Human resources: Dr. El-Bakry left the project in July 1994 and it is believed that a senior cell biologist should replace him. This program should have long term training in cell biology and in molecular biology to ensure continuity in the future. This has not been done because of lack of adequate funding but should be developed in the second phase of the project.

Intellectual properties: The different strategies that we use in this project are either covered by a patent belonging to a US company, or being filed by our team. In case of commercialization and depending on the strategy used, this will have to be sorted out except if we manage to develop our strategies.

Linkages to crop improvement/Commercialization: This project is too young to consider these aspects, which will have to be considered at the end of the second phase.

Publications

Refereed Publications, where the ABSP support has been acknowledged:

Padidam, M., Beachy, R. N., & Fauquet, C. M. (1995). Classification and identification of geminiviruses using sequence comparisons. Journal of General Virology, 76, 249-263.

Padidam, M., Beachy, R. N. & Fauquet, C. M. (1995b). Tomato leaf curl geminivirus from India has a bipartite genome and coat protein is not essential for infectivity. *Journal of General Virology* **76**, 25-35

Padidam, M., Beachy, R. N., & Fauquet, C. M. (1996). Role and function of the AV2 protein of the tomato leaf curl geminivirus. Virology, 224: 390-404.

Padidam, M., Maxwell, D. P. & Fauquet, C. M. (1997). A proposal for naming geminiviruses. *Archives of Virology* **142**, 2553-2562.

Padidam, M., Beachy, R., & Fauquet, C. (1998). Bacteriophage M13 ssDNA binding protein complements ssDNA accumulation of tomato leaf curl virus and interferes with viral movement. Submitted to L. Virology.

Padidam, M., Reddy, V. S., Beachy, R. N. & Fauquet, C. M. (1998). Molecular characterization of a plant mitochondrial chaperone GrpE. *Plant Molecular Biology* **Submitted**.

Padidam, M., Sawyer, S. & Fauquet, C. M. (1998). Recombination is frequent in geminiviruses and may

be promoting virus emergence. *Science* **Submitted**.

Published abstracts, unpublished abstracts, or posters presented at conferences and workshops related to ABSP research:

Abdallah, N. A., Aref, N. M., Fauquet, C. M., Madkour, M. A., & Beachy, R. N. (1993). Nucleotide sequence and genome organization of an infectious DNA clone of tomato yellow leaf curl virus isolated from Egypt, Glasgow, UK - 8-13 August 1993.

Fauquet, C. M., Padidam, M., & Beachy, R. N. (1994). Sequence comparison of geminiviruses can be helpful for diagnostic and taxonomy. Paper presented at the American Society for Virology - 13th annual meeting, Madison, Wisconsin - 9-13 July 1994.

Fauquet, C. M., Padidam, M., & Beachy, R. N. (1994). Molecular characterization of a tomato leaf curl geminivirus from India. Paper presented at the American Society for Virology - 13th annual meeting, Madison, Wisconsin - 9-13 July 1994.

Fauquet, C. M., Padidam, M., Sangare, A., Deng, D., Fux, C., El-Leithy, S., & Beachy, R. N. (1994). Identification, classification and control of geminiviruses by genetic engineering. Paper presented at the The Sixth Annual Society of Fellows Research Symposium, The Scripps Research Institute, La Jolla, California - 27 May 1994.

Gonzalez de Schöpke, A., Cardenas, L., El Wahed, M., El Bakry, M., Madkour, M. A., Beachy, R. N., & Fauquet, C. M. (1994). *Agrobacterium*-Mediated Transformation of Tomato. Paper presented at the ILTAB Mid-Term Review, TSRI, La Jolla, CA - 28-30 April 1994.

Padidam, M., Beachy, R. N., & Fauquet, C. M. (1994). Tomato Leaf Curl Virus from India (TLCV-In) has a Bipartite Genome. Paper presented at the ILTAB Mid-Term Review, TSRI, La Jolla, California - 28-30 April 1994.

Padidam, M., Beachy, R. N., & Fauquet, C. M. (1994). Identification, Classification and Diagnosis of Geminiviruses. Paper presented at the ILTAB Mid-Term Review, TSRI, La Jolla, California - 28-30 April 1994.

Padidam, M., Beachy, R. N., & Fauquet, C. M. (1994). Comparison of sequences allow precise diagnostic and classification of geminiviruses. Paper presented at the 1st International Symposium on Geminiviruses, Almeria, Spain, 14-17 September 1994.

Padidam, M., Gonzalez de Schöpke, A., El Leithy, S., Abdallah, N., Aref, N., Beachy, R. N., & Fauquet, C. M. (1994). Engineering Resistance against Tomato Yellow Leaf Curl Virus (TYLCV)-Egypt. Paper presented at the ILTAB Mid-Term Review, TSRI, La Jolla, California - 28-30 April 1994.

Beachy, R. N., Padidam, M., Sangare, A., & Fauquet, C. M. (1995). Viral pathogenesis and pathogen derived resistance: expectations and limitations for control of geminiviruses. Paper presented at the USA-Mexico Symposium, Cocoyoc, Mexico, 5-9 November 1995.

Padidam, M., Beachy, R. N., & Fauquet, C. M. (1995). AV2 ("Pre Coat) protein of tomato leaf curl virus - India (ToLCV-In) is required for normal levels of viral DNA accumulation and symptom development. Paper presented at the Biology and Molecular Epidemiology of Geminiviruses Symposium, Westward Look Resort, Tucson, Arizona - 3-8 June 1995.

Gonzalez de Schopke, A., Beachy, R. N., & Fauquet, C. M. (1996). *Agrobacterium*-mediated transformation in tomato. Paper presented at the ILTAB Review 1991-1996, TSRI, La Jolla, CA. - 21-22 March 1996.

Padidam, M., Beachy, R. N., & Fauquet, C. M. (1996). Classification and identification of geminiviruses. Paper presented at the ILTAB Review 1991-1996, TSRI, La Jolla, CA. - 21-22 March 1996.

Padidam, M., Beachy, R. N., & Fauquet, C. M. (1996). AV2 protein is required for efficient viral movement and coat protein regulates ssDNA accumulation in tomato leaf curl virus. Paper presented at the ILTAB Review 1991-1996, TSRI, La Jolla, CA. - 21-22 March 1996.

Padidam, M., Beachy, R. N. & Fauquet, C. M. (1994). Comparison of sequences allow precise diagnostic and classification of geminiviruses. In *1st International Symposium on Geminiviruses*. Almeria, Spain, 14-17 September 1994.

Padidam, M., Beachy, R. N. & Fauquet, C. M. (1997). Production of resistant plants to geminiviruses by expressing mutated pre-coat proteins. In *ILTAB/TSRI-ORSTOM*. USA.

Padidam, M., Chatterji, A., Beachy, R. N. & Fauquet, C. M. (1998). Role of AV2, BV1 and BC1 proteins in tomato leaf curl virus movement. In *2nd International Workshop on Bemisia and Geminiviral Diseases*. San Juan, Puerto Rico - 7 to 12 June 1998.

Padidam, M., Sawyer, S. & Fauquet, C. M. (1998). Genetic recombination is frequent among geminiviruses. In *2nd International Workshop on Bemisia and Geminiviral Diseases*. San Juan, Puerto Rico - 7 to 12 June 1998.

Patents:

A provisional filing about the discovery of a possible way to control geminiviruses in general and TYLCV-Eg in particular has been filed, waiting for the transgenic work to be completed. If the transgenic work confirms the transient essays a patent would be filed.

Biopesticide Development

Principle Investigator

Dr. Lee Bulla, University of Texas at Dallas (previously University of Wyoming)

Overall Project Goal

Determine the molecular mechanism(s) of insect resistance to the insecticidal toxins (Cry toxins) of *Bacillus thuringiensis*.

Summary of Accomplishments

Cry toxin binding region studies of BT-R_1 isolated from the tobacco hornworm (*Manduca sexta*).

The binding region for the Cry1Ab toxin of *Bacillus thuringiensis* was identified and characterized. Its main features and properties are summarized below.

- BT-R_1 (210 kDa), is a single transmembrane cadherin located in the midgut epithelium of the tobacco hornworm, *M. sexta* (Fig. 1).
- It has a high binding affinity (K_d ~ 1nM) for the Cry1A toxins of *B. thuringiensis.*
- A contiguous 169-amino acid sequence adjacent to the MPED binds the Cry1Ab toxin.
- The purified toxin-binding fragment acted as an antagonist to Cry1Ab toxin by blocking the binding of toxin to the tobacco hornworm midgut and inhibiting insecticidal action.
- Exogenous Cry1Ab toxin bound to intact COS-7 cells expressing BT-R_1 cDNA, subsequently killing the cells.
- Recruitment of BT-R_1 by *B. thuringiensis* indicates that the bacterium interacts with a specific cell adhesion molecule during its pathogenesis.

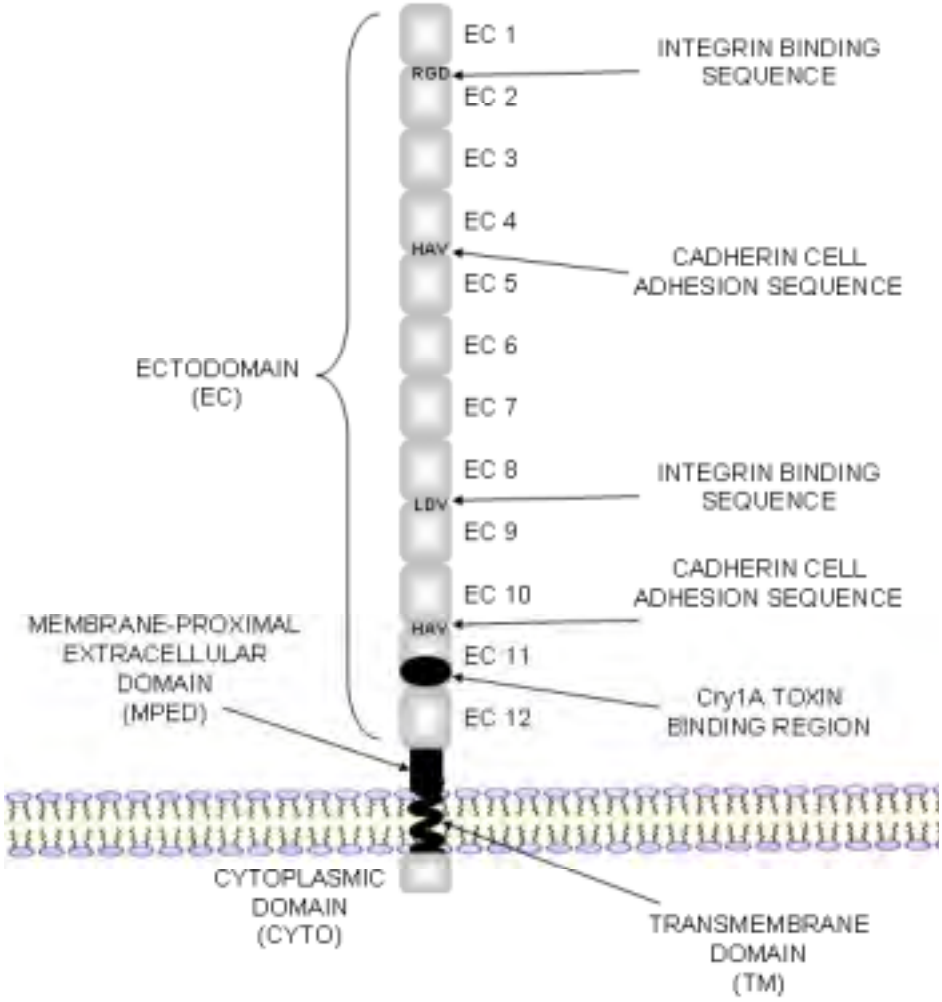


Fig. 1. Structure model of bt-r_1

Studies on the proteolytic cleavage of BT-R_1 during development of *Manduca sexta* larvae

The development of tobacco hornworm larvae and the accompanying changes in the abundance of BT-R_1 concomitant to specific cleavage of the molecule in the different developmental stages (instars) of the larvae were studied.

- Expression of BT-R_1, which varies during larval development, correlates with abundance of the protein and with the differential cleavage of the molecule at each developmental stage.
- The cleavage of BT-R_1 is calcium-dependent and, consequently, Ca^{2+} directly influences the structural integrity of BT-R_1.
- Removal of calcium ions by chelating agents promotes cleavage of the BT-R_1 ectodomain, resulting in formation of fragments that are similar to those observed during larval development (Fig. 2).
- Partial purification of proteins from brush border membrane vesicles (BBMV) by gel filtration

chromatography hinders the cleavage of BT-R_1 in the presence of ethylenediaminetetra-acetic acid and ethylene-glycol-bis(2-aminothyl ether)-N,N,N,N′-tetraacetic acid, indicating that there is specific proteolytic activity associated with the BBMV.

- The specific proteolytic cleavage of BT-R_1 not only alters the integrity of BT-R_1 but it most likely is implicated in cell adhesion events during differentiation and development of *M. sexta* midgut epithelium as well.

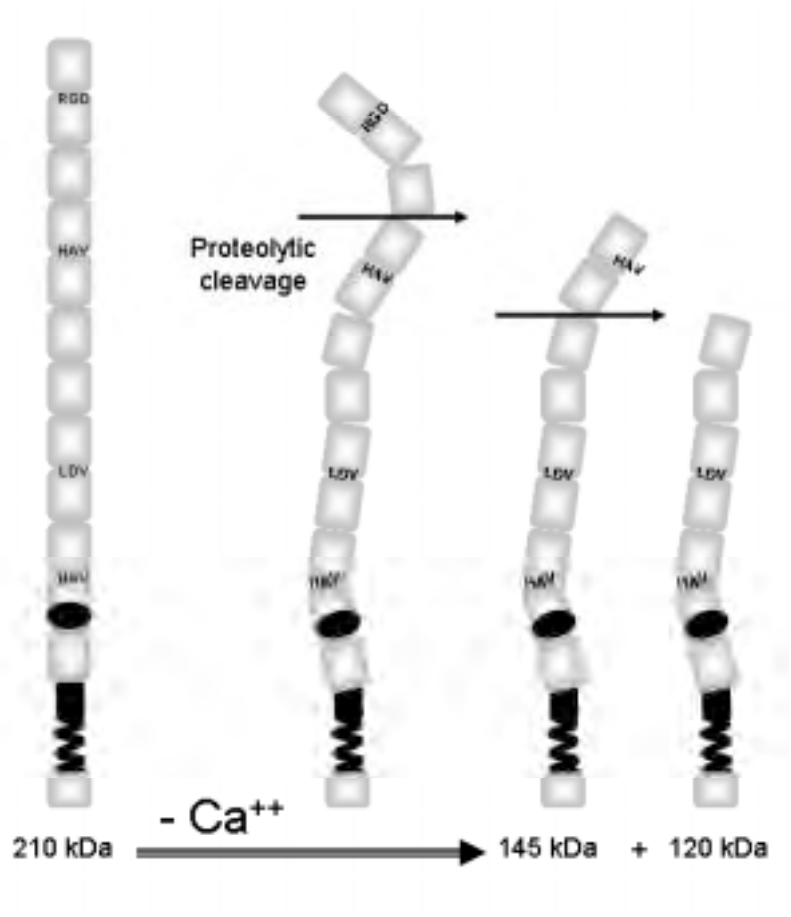


Fig. 2. Model of calcium-dependent cleavage of bt-r_1

Studies on the expression of a midgut-specific cadherin BT-R_1 during the development of *Manduca sexta* larva

Critical observations based on this investigation are listed below.

- The *btr-1* gene of *Manduca sexta* (GenBank AF319973) encodes a cadherin, BT-R_1 (210-kDa), which contains 12 ectodomain modules in association with a number of motifs potentially involved in interactions with cadherin and integrin.
- BT-R_1 is localized exclusively in the midgut epithelium (Fig. 3) and it was demonstrated using BBMV proteins from tissues of fifth instar *M. sexta* larvae separating them by SDS-PAGE and detecting BT-R_1 by ligand blotting with BT-R_1 antiserum (A) and with ^{125}I-Cry1Ab (B). Lane 1, Malpighian tubules; lane 2, trachael tubes; lane 3, fat body; lane 4, peritrophic membrane; lane 5, foregut; lane 6, anterior midgut; lane 7, middle midgut; lane 8, posterior midgut; lane 9, hindgut.

- The amount of BT-R_1 protein increases dramatically during larval development, paralleling accumulation of its mRNA.
- The 5′-UTR of the *btr-1* gene contains sequence motifs that most likely recruit specific transcription factors, particularly, those that determine posterior patterning and that control intestinal cell proliferation, differentiation and identity during development.
- The increase in abundance of BT-R_1 is required to support not only the differentiation of the epithelial cells but also the establishment of physiological function and structural integrity of the midgut during larval development in *M. sexta*.
- BT-R_1 is essential to larval midgut epithelial organization during rapid cell proliferation and tissue growth in *M. sexta* because disruption of such organization and functionality occasioned by the binding of the Cry1A toxins of *B. thuringiensis* to BT-R_1 causes death to the insect.

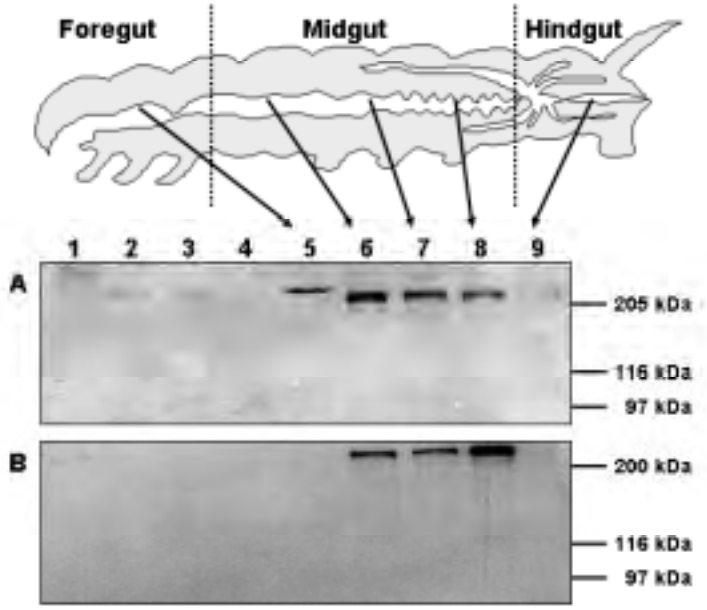


Fig. 3. Midgut-specific expression of bt-r_1

Insect resistance studies on the Colarado potato beetle (*Leptinotarsa decemlineata*). The protease activity profiles and toxin-binding capacities in the midgut of a strain of Colorado potato beetle that has developed resistance to the Cry3Aa toxin of *B. thuringiensis* subsp. t*enebrionis* were investigated. The following points represent the salient features of the study.

- The structural integrity of the midgut tissue in the toxin-resistant (R) insect was retained whereas the same tissue was devastated by toxin action in the susceptible (S) strain (Fig. 4)
- Function-based activity profiling using zymographic gels showed specific proteolytic bands present in midgut extracts and brush border membrane vesicles (BBMV) of the R strain not apparent in the S strain.
- Aminopeptidase activity associated with insect midgut was higher in the R strain than in the S strain.
- Enzymatic processing of toxin did not differ in either strain and, apparently, is not a factor in resistance.

- BBMV from the R strain bound ~60% less toxin than BBMV from the S strain whereas the kinetics of toxin saturation of BBMV was 30 times less in the R strain than in the S strain.
- However, homologous competition inhibition binding of ^{125}I-Cry3Aa to BBMV did not reveal any differences in binding affinity (K_d ~ 0.1 μM) between the S and R strains.
-

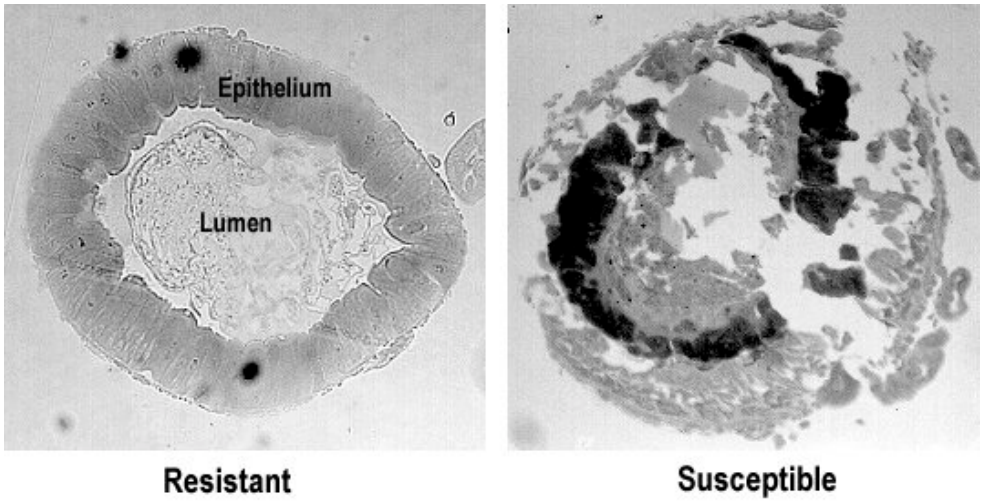
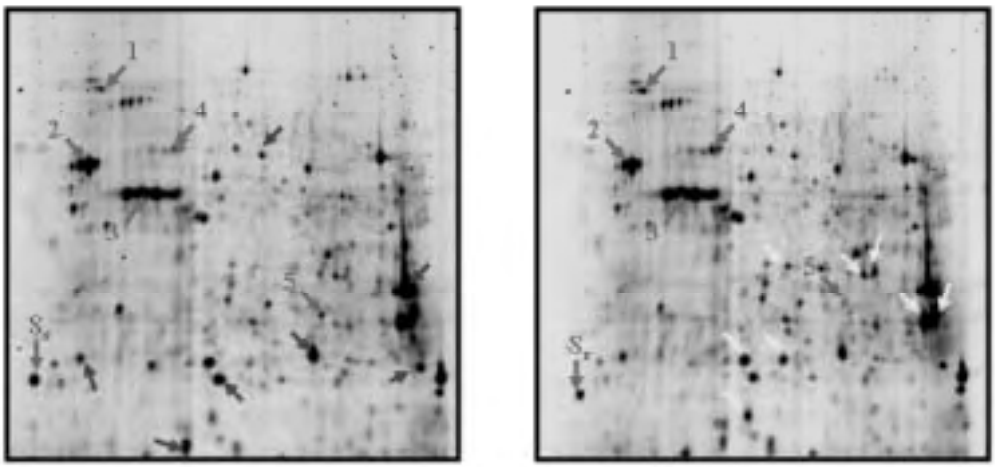


Fig. 4. Electron micrographs of cross sections of midguts of resistant & susceptible colorado potato beetle larvae treated with cry1ab toxins of *bacillus thuringiensis*

Insect resistance studies on the Indianmeal moth (*Plodia interpunctella*).
Studies on insect resistance to the entomocidal Cry1Ab toxin of *Bacillus thuringiensis* by the Indianmeal moth (IMM) were accomplished by examining alterations in its larval gut proteome (Fig. 5). The results of this experimental approach are highlighted below.

- There were significant increases in the levels of an aminopeptidase and a vacuolar ATPase in resistant (R) larvae compared to susceptible (S) larvae; no changes were detected in the levels of mitochondrial ATPsynthase or actin; a significant decrease in a chymotrypsin-like proteinase occurred in the R larvae (Fig. 6).
- Two unique unidentified proteins (“shift” proteins) were detected in the S and R insect colonies, which may be potential markers for S and R populations of the Indianmeal moth.
- IMM resistance to the Cry1Ab toxin of *Bacillus thuringiensis* is multifactorial and involves changes in the composition or modification of various proteins or both.
- The multifactorial response can be detected by comparison of the proteomic expression profiles in the S and R strains of the IMM (Fig. 5).

Susceptible **Resistant**



Gray arrows indicate proteins in S strain that show higher accumulation than the R strain.

White arrows indicate proteins in the R strain that show higher accumulation than the S strain.

S_s – susceptible "shift" protein
S_r – resistant "shift" protein
1 – aminopeptidase
2 – mitochondrial ATP synthase
3 – actin
4 – vascular ATPase
5 – chymotrypsin-like protease

Fig. 5. Proeomic analysis of imm strains susceptible & resistant to cry1ab toxin of *bacillus thuringiensis*

Protein I.D.	M.W. (kDa)	p*I*	Masses matched (%)	Spot volume abundance (average ratio; S/R)
Aminopeptidase	115.5	4.6	21	-1.2
Mitochondrial ATP *synthase*, β chain	56.2	5.1	18	1.0
Actin	42.2	5.5	20	1.0
Vacuolar ATP *synthase*, *subunit* B	55.2	5.3	25	-1.9
Chymotrypsinogen-like protein	30.4	8.9	24	6.0

S_s – susceptible "shift" protein
S_r - resistant "shift" protein

Fig. 6. Relative amounts of specific proteins in the Indianmeal moth gut proteome

Significance and Impact of the Research Accomplished

Cry toxin binding region studies of BT-R_1 isolated from the tobacco hornworm (*Manduca sexta*).
Targeting host cadherins and other cell junction molecules may be representative of those bacteria that disrupt or evade epithelial barriers in their hosts. Evidently, the pathogenicity of *B. thuringiensis* shares a common mechanism(s) with other bacterial pathogens that target host tissues. Many of the open reading frames (ORFs) in the *Bacillus anthracis* virulence plasmid pXO1 show 80 and 98% similarity to genes in isolates of closely related *Bacillus* species, including *B. cereus* and *B. thuringiensis*. However, it remains to be determined whether there are commonalities among *B. thuringiensis*, *B. anthracis* and *B. cereus*, which have a high degree of genetic relatedness and whose toxins target cell-surface receptors). Nevertheless, recruitment of BT-R_1 by *B. thuringiensis* to overpower a susceptible host is a model system to study bacterial pathogenesis and to examine co-evolutionary relationships among insects and entomopathogens. Publication 5 listed below is a result of these studies.

Studies on the proteolytic cleavage of BT-R_1 during development of *Manduca sexta* larvae.
Specific proteolytic cleavage of cadherin ectodomains is an important aspect of cell regulation and cell interactions. Developmental and tissue-specific expression of BT-R_1 in the midgut of *M. sexta* larvae supports our hypothesis that the cleavage of the BT-R_1 ectodomain is important to specific cell

interactions in the midgut epithelium. The midgut consists of highly structured epithelial cells that are organized in a characteristic pattern. During larval development (including growth and larval-larval molts), the organization of *M. sexta* midgut cells undergo dramatic arrangements to support cell proliferation as well as structural and functional integrity of the tissue indicating involvement of dynamic cell-cell adhesion events in this insect as is true for all metazoan tissues. Some of the activities associated with cadherins involve not only cell-cell adhesion events but activation of specific intracellular signaling pathways as well Thus, increased proteolysis of cadherins, which as been implicated in various morphogenetic events, affects both cell-cell contact and cell-cell communication. The increase in BT-R_1 proteolysis that occurs, especially in the fifth instar, precedes the destruction of larval midgut epithelium prior to replacement by pupal epithelium, reflecting turnover of this particular cadherin at the end of larval development. Isolation and characterization of the proteolytic activity related to the cleavage of the cadherin receptor BT-R_1 as well as determination of the cleavage sites on the molecule are import aspects for future investigations. Publication 3 listed below is a result of these studies.

Studies on the expression of a midgut-specific cadherin BT-R_1 during the development of *Manduca sexta* larva.

BT-R_1 provides selective adhesive properties and dynamic multi-cellular interactions in the larval gut and is involved in the innate immunity of the tobacco hornworm. Because various cadherins differ in their expression patterns and because dynamic changes occur in cell adhesion during development, it is important to ascertain whether BT-R_1 is indispensable to *M. sexta* larval morphogenesis. Modulation of cell adhesion and cell communication is critical to accommodate the changes related to cell differentiation in *M. sexta* midgut. The features of the cadherin receptor BT-R_1, its expression in *M. sexta* larvae and the molecular interactions of this particular molecule may provide valuable insight into tissue development and organ structuring in this insect as well as in other multi-cellular organisms. Publication 2 listed below is a result of these studies.

Insect resistance studies on the Colarado potato beetle (*Leptinotarsa decemlineata*).

The results indicate that resistance by the CPB to the Cry3Aa toxin correlates with specific alterations in protease activity in the midgut as well as with decreased toxin binding. These features reflect adaptive responses that render the insect refractory to toxin action, making this insect an ideal model to study host innate responses and adaptive changes brought on by bacterial toxin interaction. Publication 6 listed below is the result of these studies.

Insect resistance studies on the Indianmeal moth (*Plodia interpunctella)*.

Comparative proteomic analysis of susceptible and resistant insects promotes understanding of the innate responses and adaptive changes in the insect gut epithelium and their functional involvement in rendering immunity to the Cry toxins of *B. thuringiensis*. Such analysis of the IMM and other insects that exhibit increased potential to resist Cry toxins should reveal how insects adapt or develop resistance to survival challenges. Assessment of proteomic differences, along with biochemical and genetic changes associated with insect resistance to *B. thuringiensis*, will be helpful to develop strategies for circumventing the evolution of resistance in target insect populations and in managing the long-term efficacy of biopesticides based on *B. thuringiensis*. Publication 1 listed below is the result of these studies.

Publications

Candas, M., O. Loseva, B. Oppert, P. Kosaraju and L. A. Bulla, Jr. 2002. Insect Resistance to the Entomocidal Cry1Ab Toxin of *Bacillus thuringiensis*: Alterations in the Indianmeal Moth Larval Gut Proteome. Submitted.

Midboe, E. G., M. Candas and L.A. Bulla, Jr. 2002. Expression of a Midgut-specific Cadherin BT-R_1 During the Development of *Manduca sexta* Larva. Submitted.

Candas, M., B. R. Francis, N. B. Griko, E. G. Midboe and L. A. Bulla, Jr. 2002. Proteolysis of BT-R_1: a developmentally important cadherin receptor in *Manduca sexta* epithelium. *Biochemistry*. In press.

Candas, M. and L. A. Bulla, Jr. Insecticides, microbial. 2002. *Encyclopedia of Environmental Microbiology*. Vol. 1 (ed. G. Bitton). John Wiley & Sons, Inc. NY, pp. 1709-1717.

Dorsch, J. A., M. Candas, N. B. Griko, W. S. A. Maaty, E. G. Midboe, R. K. Vadlamudi and L. A. Bulla Jr. 2002. Cry1A Toxins of *Bacillus thuringiensis* Bind Specifically to a Region Adjacent to the Membrane-proximal Extracellular Domain of BT-R_1 in *Manduca sexta*: Involvement of a Cadherin in the Entomopathogenicity of *Bacillus thuringiensis. Insect Biochemistry and Molecular Biology*. 32:1025-1036.

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

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

Meng, J., M. Candas, T. P. Keeton and L. A. Bulla, Jr. 2001 Expression in *Spodeptera frugiperda* (Sf21) Insect Cells of BT-R_1, a Cadherin-related Receptor from *Manduca sexta* for *Bacillus thuringiensis* Cry1Ab Toxin. *Protein Expression and Purification*. 22:141-147.

Keeton, T. P., B. A. Francis, W. S. A. Maaty and L. A. Bulla, Jr. 1998. Effects of midgut-protein-preparative and ligand binding procedures on the toxin binding characteristics of BT-R_1, a common high-affinity receptor in *Manduca sexta* for Cry1A *Bacillus thuringiensis* toxins. *App. Environ. Microbiol.* 64:2158-2165.

Collaborations with Egyptian Scientists and Transferal of Results to Egypt

Drs. M. Ibrahim and W. S. A. Maaty received their PhD's under Dr. Bulla's direction and, since, have returned to Egypt where they hold important research positions in the Agricultural Genetic Engineering Institute (AGERI), Agricultural Research Center, Egyptian Ministry of Agriculture and Land Reclamation. Dr. Ibrahim is in charge of a research program on microbial insecticides at AGERI and Dr. Maaty is leading investigations in genomics and proteomics, focusing on cell surface molecules and their involvement in signal transduction pathways.

Dr. Ibrahim was involved in the insect resistance studies summarized above and was co-senior author of publication 6 and is preparing a second manuscript for publication that focuses on the characterization of one of the important Cry toxins of *Bacillus thuringiensis*. Dr. Maaty was involved in the Cry toxin binding studies (publication 5) and is preparing a second manuscript for publication that involves similar kinds of results for the pink bollworm.

Much of the technology for characterizing Cry toxin binding receptors in insects for studying resistance to *B. thuringiensis* has been transferred to AGERI and is being applied to the cotton leafworm and the potato tuber moth, both serious agricultural pests in Egypt. All of this technology and information has direct bearing on the two insects. Because many of the results gained in this program are first-time discoveries, these two Egyptian agricultural scientists along with their colleagues at AGERI (Drs. Salah

Moustoufa and Gamel Osman who also studied with Dr. Bulla) will have a head start, both scientifically and practically, to address some critical problems related to insect resistance to *B. thuringiensis*.

AGERI now has a team of scientists, all of which were trained in Dr. Bulla's laboratory and under the sponsorship of this CUB program, who will be addressing these concerns, among others. All of the results obtained in Dr. Bulla's laboratory on the two model insect systems, tobacco hornworm (*Manduca sexta*) and the Colorado potato beetle (*Leptinotarsa decemlineata*) will be utilized to examine the cotton leafworm and the potato tuber moth in Egypt. Much of the information gained in the studies funded by USAID was obtained directly by Egyptian students and scientists studying with Dr. Bulla. This team now is prepared to tackle the potentially serious problem of insect resistance to *B. thuringiensis* before it becomes a dilemma for Egyptian agriculture.

Egyptian Research Publications

Maize

Development of efficient regeneration and transformation system for Egyptian maize inbred lines. A poster presented at the 6th International Conference of Plant Molecular Biology. June 18-24, 2000. Quebec, Canada.

Shireen K. Assem, July (2001): Callus production and plant regeneration in Egyptian maize genotypes. Arab Journal of Biotechnology. July, 2001 (In press)

Shireen K. Assem, Ebtissam H.A. Hussein, Hanaiya A. El-Itriby, Fathy M.Abd El-Galil, and Magdy A. Madkour. (July 2001).The competence of maize shoot meristems for transformation of Egyptian maize inbred lines. Arab Journal of Biotechnology. July, 2001 (In press)

Shireen K. Assem, Hanaiya A. EL-Itriby, Ebtissam H.A. Hussein, Fathy M. Abd El-Galil and Magdy A. Madkour. The regeneration and Transformation of Egyptian maize inbred lines via immature embryo culture and biolistic particle delivery system. Submitted to: *In vitro* Plant Cellular and Developmental Biology. (May 2001).

F.A. Abdel-Tawab, Hanaiya A. El-Itriby, A. Bahieldin. M.A. Yossef and M.A. Madkour. Regeneration and genetic transformation of different genotypes in maize (Zea mays L.). Egypt. J. of Genet. And Cytol. Vol 30: 1-21, 2001.

El-Itriby H. A., S. K. Assem, E. H. A. Hussein; and M. A. Madkour (2001). The competence of maize shoot meristems for transformation of Egyptian maize inbred lines. Arab Journal of Biotechnology, 4(2): 149-162.

Assem S. K (2001). Callus production and plant regeneration in Egyptian maize genotypes. Arab Journal of Biotechnology, 4(2): 149-162.

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

Tomato

Abdallah, N, A. and Aref, N. M. (1994). Isolation and characterization of whitefly transmitted virus-like particles in sugar beet. Egyptian Journal of Genetics and Cytology. Vol. 23:179-199.

Aref, N. M.; Abdallah, N.A.; Allam, E.K. and Madkour, M.A. (1994). Use of polymerase chain reaction and radiolabled specific probe to identify tomato yellow leaf curl virus from infected plants. The Egyptian Phytopathological Society VIIth Congress of Phytopathology, pp.93-109.

Abdallah, N.A. (1995). Quantitation of tomato yellow leaf curl virus (TYLCV) genome by quantitative competitive polymerase chain reaction in tomato and tobacco. Journal of Union Arab Biologists Cairo. Vol. 2(B):15-25.

Aref, N. M. and Abdallah N, A. (1995). Virus like particles associated with leafroll disease in sugar beet and tomatoes unusual ssDNA component. Egyptian Journal of Genetics and Cytology. Vol. 24:107-135.

Aref, N.M.; Abdallah, N.A.; Tohamy, A.M. and Madkour, M.A. (1995). Genomic and serological properties of tomato yellow leaf curl geminivirus isolated in Egypt. 6th Nat. Conf. of Pest. & Vegetables & Fruits in Egypt, pp: 339-368.

Abdallah, N.A.; Aref, N.; Idreiss, M. ; Brown, J. and Madkour, M. (1995). Molecular variation among

whitefly-transmitted geminiviruses in different hosts from Egypt. The International Geminivirus Conference, Tucson, AZ, June 3-8. p13-21.

Abdallah, N.A.; Abou-Salha, A. and Soliman M. (1996). Functional studies of complementary sense promoter (replicase promoter) from tomato yellow leaf curl virus: Evidence for transcription capability in non-host plants. J. Phytopathology, 144: 251-256.

Idriss, M.; Abdallah, N.; Aref, N. Haridy, G. and Madkour, M. (1997). Biotypes of the castor bean whitefly Trialeurodes ricini (Misra) in Egypt: Biochemical characterization and efficiency of geminivirus transmission. J. Appl. Ent., 121: 501-509.

Idriss, M.; Abdallah, Nand Madkour, M. (1998). Does the castor bean whitefly (CBW) Trialeurodes ricini (Misra) transmit geminivirus? 2nd International Workshop on Bimisia and geminiviral disease. June 7-12, San Juan, Puerto Rico; P-14.

Abdallah, N.A.; Gafer, D.E.; Malash, N.M., Salama, S.A. and Madkour, M. (1998). Evaluation of different factors influencing transformation and regeneration frequency of tomato (Lycopercian esculantum L.). Egyptian Journal of Genetics and Cytology. Vol. 27: 221-244.

Hafez, E. E., N. A. Abdallah; S. M. El-Dohlob and M. A. Madkour (1999). Biological study on sugarcane streak virus (SSV) in Egypt. Egypt. J. Phyt.

Abdallah, N.A.; Fauquet, C.M.; Beachy, R. N. and Madkour, M. (2000). Cloning and constructing infectious clones of an Egyptian isolate of tomato yellow leaf curl virus. Arab J. Biotech., 3(1): 35-54.

Shamloul, A.M.; Abdallah, N.A.; Madkour, M.A. and Hadidi, A. (2001). Sensitive detection of the Egyptian species of sugarcane streak virus by PCR-probe capture hybridization (PCR-ELISA) and its complete nucleotide sequences. J. of Virological Methods, 92: 45-54.

Potato

Osman, Y. A., T. M. Naser El-Din, M. Ibuahim, E. A. Metry and M. A. Madkour. 2001. Production of Genetically Engineered Potato /resistant to Potato Tuber Moth. Under publication and will be presented at the Nineth National Conference of Pests and Diseases of Vegetables and Fruits in Egypt (Ismailia, November 2001)

Douches, D.S., W. Pett, F. Santos, J. Coombs, E. Grafius, W. Li, E. A. Metry , M. Madkour and T. Nasr El-Din. 2003. Field and Storage Testing Bt-Potatoes for Resistance to Potato Tuber Moth (Lepidoptera: Gelichiidae). J. Econ. Entomol. (accepted Dec 2002).

Squash

Said M. Khalil, Atef S. Sadik, Hamdy El-Doweny and Magdy A. Madkour (1999). Production of transgenic squash plants resistant to zucchini yellow mosaic potyvirus. Arab Journal of Biotechnology 2(I): 27-44.

S.M.Khalil, M.A.Badawl, 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(I):87-96.

Publications under preparation

Agrobacterium-mediated transformation of cantaloupe to confer resistance to zucchini yellow mosaic potyvirus. Gihan M. Hosny, Atef S. Sadik, Hamdy El-Doweny and Magdy A. Madkour.

Field evaluation of virus resistant transgenic squash lines transfared with the ZYMV cp gene. Atef S. Sadik, Said M. Khalil, Hamdy El-Doweny and Magdy A. Madkour.

Egyptian Participants in ABSP Training 1995-2002

Name	Dates	Location	Purpose
Ms. Shireen Assem	1995, 1997	ICI Seeds, Iowa	Maize transformation with Bt genes
Mr. Mohamed Tawfik	1999-2003	Michigan State University (Dr Rebecca Grumet)	Ph.D. student working on engineering increased salinity and drought resistance in cucumber.
Mr. Mohamed Eid Saad	1997-1998	Pioneer Hi-Bred, Des Moines, Iowa	Identification and cloning of promoters to drive the expression of Bt insecticidal genes
Mr. Mohammed Abd el Wahed	1997-1998	Pioneer Hi-Bred, Des Moines, Iowa	Bt maize transformation.
Mr. Haridy Osman	1997-1998	Pioneer Hi-Bred, Des Moines, Iowa	Field and laboratory Bt maize bioassays
Mr. Salah Mousafa	1997-1998	Pioneer Hi-Bred, Des Moines, Iowa; University of Wyoming (Dr. Lee Bulla)	Bt-receptor molecular biology and protein chemistry.
Dr. Yehia Osman	1997-1998	Pioneer Hi-Bred, Des Moines, Iowa; University of Wyoming (Dr. Lee Bulla)	Bt-receptor molecular biology and protein chemistry.
Drs. M. Ibrahim		University of Wyoming (Dr. Lee Bulla)	Bt-receptor molecular biology and protein chemistry.
Dr W. S. A. Maaty		University of Wyoming (Dr. Lee Bulla)	Bt-receptor molecular biology and protein chemistry.
Drs. Salah Moustoufa		University of Wyoming (Dr. Lee Bulla)	Bt-receptor molecular biology and protein chemistry.
Dr. Gamel Osman		Pioneer Hi-Bred, Des Moines, Iowa; University of Wyoming (Dr. Lee Bulla)	Bt-receptor molecular biology and protein chemistry.

Egyptian Participants in ABSP Training Programs 1995-2002

PARTICIPANTS	*Affiliation*
Internship for Risk Assessment & Field-Testing of Transgenic Plants (USDA, MSU, Virginia Tech) 1995	
Dr Ahmed Wally	AGERI
MSU Biosafety Internship Program 1996	
Dr. Taymour Nasr El-Din	AGERI
Mr. Khaled Essam	AGERI
MSU Integrated Pest Management Course 1996	
Mr. Ahmed Mohamed Ahmed	AGERI
Mr. Gamal Ebrahim Haridy Osman	AGERI
MSU IPR Course 1997	
Mr. Taymour Nasr El Din	AGERI
Dr. Abdel M. Moustafa	USAID/Cairo
Mr. Osama Saad Sayed Hassan	AGERI
MSU IPR Course 1998	
Mr. Mohamed Abd El Wahed	AGERI
Mr. Ali Mohamed Abdel Hamid Ahmed	Egyptian Export Promotion Center
Dr. Nabila Mohamed Gad Attia	Egyptian Export Promotion Center
Mr. Gamal Eissa Attya	Central Administration for Seed Testing & Certification
Dr. Ahmed Abd El-Khalik El Behery	AGERI
Mr. Mohamed Rizk Enan	AGERI
Dr. Gharib Gad El Karim	AGERI
Mr. Sayed Abdel Mawgoud Mahmoud	Central Administration for Seed Testing & Certification
Mr. Mohamed Abdel Moneim Mohamed Morsy	Central Administration for Seed Testing & Certification
Mr. Ehsan M. Tewfik	Egyptian Export Promotion Center
Mr. Mohamed Ibrahim Abdel Wahed	Egyptian Export Promotion Center
MSU IPR Course 1999	
Amr Mohamed Mahmoud Ageez	AGERI
MSU IPR Course 2000	
Mr. Motaz A. Moniem	AGERI

MSU IPR Course 2002	
Dr. Mohiey Batanouny	Agriculture Technology Utilization and Transfer (ATUT) Project
Ms. Samia S. Mostafa	Academy of Scientific Research and Technology - Patent Office
Mrs. Janet Ibrahim Youssef	Academy of Scientific Research and Technology - Patent Office
MSU Food Safety Course 1999	
Taymour Nasr El-Din	AGERI
Hisham El-Sheshtawy	AGERI
Dr. Badaweya Hamza	Food Technology Research Institute
MSU Food Safety Course 2000	
Dr. Badaweya Hamza	Food Technology Research Institute
Egypt IPR Course 2001	
Safwat Khawaga	AGERI
Mahmoud Saleh	AGERI
Gamal Zaza	AGERI
Atef Wahab	AGERI
Salah Khalil	AGERI
Ahmed Ashoub	AGERI
Essam Michael	ARC
Eid Megeed	ARC/Egypt
Ismail Abdel-Hamid	ARC-AGERI
Angail Saad	Control Admin for Seed Testing
Mohamed Morsy	Control Admin. For Seed Testing
Ahmed Gawhar	Control Admin. For Seed Testing
Mohamed Sheikh	Control Admin. For Seed Testing
Mohamed Yamany	Control Admin. For Seed Testing
Samir Hassan	Control Admin. For Seed Testing
Weam Abdalla	Control Admin. For Seed Testing
Gamal Bayoumy	Control Admin. For Seed Testing
Mohamed Morsy	Control Admin. For Seed Testing
Abdallah Shafai	Sugar Crops Research Institute

Budget Information

Equipment Purchased Under 26-0240-G-00-6014-00 by MSU for AGERI, 2002

Description	PO#	Cost	Vendor
Lab Information Management System	140924	$89,990.00	Geospiza, Inc.
SpotArray 72 + ScanArray Lite	144094	$149,164.75	Perkin-Elmer
Typhoon Workstation 9410 + software	144095	$131,660.00	Amersham Biosciences Corp.
GelPix	144465	$111,953.00	Genetix
Automated Benchtop Pick & Grid System	144466	$122,064.50	Genetix
Multiprobe HT II Ex	144537	$113,814.00	Perkin-Elmer
Thermo-Savant, Speedvac.	144951	$10,840.00	ThermoSavant
ABI 3100	144566	$166,600.00	Applied Biosystems Division
Multifuge 35-4	145711	$14,067.06	Kendro Lab
Shipping & Handling		$5,846.69	
	Total	$916,000.00	

Commercialization and Utilization of Biotechnology Final Budget Summary

Grant No. 263-0240-G-00-6014-00

Period of Award: 10/15/95 – 09/30/02

MSU Account Nos. 61-2828 thru 61-2833

Line Item	Total Obligated Fund	Expended Year 7 to date	Total Expenditures
Management	$396,433.30	$25,304.47	$396,433.30
Commercialization of Products/IPR/Biosafety	$1,353,574.76	$125,117.83	$1,353,574.76
Potato Insect Resistance/MSU	$701,266.30	$185,071.77	$701,266.30
Cucurbit Crops/Virus Resistance/MSU	$318,190.92	$36,688.08	$318,190.92
Melons & Cucumbers/Virus Resistance/CorneII	$237,076.46	$95.42	$237,076.46
Whitefly Biotypes/Arizona	$67,738.51	-0-	$67,738.51
Tomatoes/Virus Resistance/Scripps	$182,442.00	-0-	$182,442.00
Maize/Insect Resistance/Pioneer Hi-Bred	$261,890.18	-0-	$261,890.18
Bio-Pesticide Development/Wyoming	$100,552.22	-0-	$100,552.22
Abiotic Stress/Ohio State University	$110,300.00	$11,001.00	$110,300.00
Bt Receptors/Univ of Texas at Dallas	$186,027.12	$80,377.12	$186,027.12
Equipment for AGERI	$916,000.00	$916,000.00	$916,000.00
Consumables for AGERI	$134,236.23	$134,236.23	$134,236.23
Indirect Costs on Subagreements	$82,250.00	.01	$82,250.00
TOTAL	$5,047,978.00	$1,513,891.93	$5,047,978.00