

AGRICULTURAL BIOTECHNOLOGY SUPPORT PROJECT



FINAL TECHNICAL REPORT 1991 -- 2003

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Introduction

This is the final report of the Agricultural Biotechnology Support Project (ABSP). The report outlines the accomplishments and impacts of ABSP under the USAID cooperative agreement DAN-A-00-00126-00 and contract 263-0240-G-00-6014-00. It covers the period September 30 1991 through June 30 2003.

The overall goal of the first Phase of the ABSP Project (1991-1998) was:

To mutually enhance U.S. and developing country institutional capacity for the use and management of biotechnology research to develop environmentally compatible, improved germplasm.

In 1998 as part of a review of the progress of the project, a slightly revised overall goal was developed for Phase II of the project's activities (1998-2003):

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

During this phase of the project, this overall goal was addressed under two main objectives:

- 1) *The establishment of a policy framework in developing countries and transition economies that promotes the use, management and commercialization of biotechnology by both host country and multinational agribusiness and research institutions.*
- 2) *Improvement of marketed crops through strategic research partnerships between the US and developing country public and private sectors.*

This report addresses the whole life of the ABSP, giving an overview of activities, achievements, impacts, lessons learned, and a brief assessment of the implications of these for future USAID projects in agricultural biotechnology.

ABSP: A Background Primer

ABSP: A Background Primer

History of ABSP

In the mid 1980's as agricultural biotechnology began to move toward commercialization in the United States, the development community began to look at the potential application of biotechnology in developing countries. At the time, much was written in the development literature about the potential benefit for food security and, conversely, the potential for economic harm due to replacement of developing country crops.

The first initiative of the U.S. Agency for International Development (USAID) in the area of biotechnology was the Tissue Culture for Crops Project (TCCP) based at Colorado State University. Research sponsored under the TCCP sought to produce crops (wheat, rice and sorghum) tolerant to an array of stresses, including salinity, drought, and acid/aluminum soil conditions. TCCP scientists and plant breeders in universities and international and national research programs developed research teams for each objective. These research programs, some of which were very successful, relied primarily upon somaclonal variation, tissue culture and *in vitro* selection to produce novel sources of genetic variation. However, these approaches were found to be limited in their ability to achieve the objectives of the project, and in 1989 USAID therefore began a review of opportunities to support biotechnology. The purpose was to identify constraints on agricultural productivity in the developing world and relevant technologies to address these constraints in the near-term future, meaning three to five years.

USAID recognized that biotechnology applications, integrated into traditional systems, had the potential to address some of the constraints to agricultural production and productivity, particularly given the need to increase production in a sustainable manner while also protecting the environment and maintaining biodiversity.

Some of the advantages of agricultural biotechnology cited by USAID included:

- ◆ The potential to increase productivity and food availability through better agronomic performance of new varieties, including resistance to pests;
- ◆ Greater stability in farm production and reduced need for expensive inputs;
- ◆ Rapid multiplication of disease free plants; and
- ◆ Diagnosis of diseases of plants.

As part of this process, USAID commissioned the National Academy of Science's National Research Council to provide recommendations for a program that would match opportunities in the U.S. and constraints in developing countries. The resulting report from the National Research Council, *Plant Biotechnology Research for Developing Countries*, served as the primary base for what was to become the Agricultural Biotechnology Support Project (ABSP). This new project was designed to bring together public sector and commercial research efforts in an integrated product-development program. The project would be awarded following peer review of proposals submitted to USAID based upon a formal Request for Applications.

The proposal review process resulted in the award by USAID of a cooperative agreement to Michigan State University in September 1991 to implement a new phase in its support for plant biotechnology. The main goal of the Agricultural Biotechnology for Sustainable Productivity (ABSP) project was ***'to mutually enhance U.S. and developing country institutional capacity for the use and management of biotechnology research to develop environmentally-compatible, improved germplasm.'***

The project was to be guided by values and principles that reflected a balance of the issues surrounding biotechnology research, both at universities and in the private sector, in the U.S. and USAID's development philosophy:

- ◆ **Sustainability.** The project should fit into the context of agricultural sustainability--that is, an agricultural system that meets rising demands for food at economic, environmental, and other social benefits consistent with improved living conditions.
- ◆ **Biosafety.** Biosafety review and regulation should be internalized in the developing countries themselves as a result of the project.
- ◆ **Intellectual property rights (IPR).** As collaboration between public and private research groups in the United States increases through the course of the project, patent protection of research should be assured so that products can be developed for public benefit.
- ◆ **Human resource development and networking.** Human capacity for biotechnology should be enhanced through doctoral and postdoctoral fellowships or other forms of training.

The importance of policy

The rationale for integrating research and technical capacity building with policy work on IPR and biosafety was based on technology transfer trends emerging in the U.S at that time. Recognizing the significant private sector role in biotechnology research, USAID sought to engage the private sector in the development process and to promote both local and international investment in developing countries. This meant addressing IPR both as a means of accessing proprietary technologies from the private sector and in considering the policy environment needed to stimulate private sector investment. Secondly, as a result of the Bayh-Dole Act of 1985, IPR was becoming a more common tool for public universities in the U.S. to promote private sector investment in development and commercialization of research technologies. Thus, in the U.S., linkages were being formed between the public and private sectors, particularly in biotechnology, and managing IPR and biosafety were becoming routine components of university research systems.

Public versus private sector research

In most developing countries, however, agricultural research is conducted almost exclusively within public sector institutes. The private sector in many developing countries is underdeveloped or poorly linked to public research institutions. Additionally, government policies may not encourage investment in research-intensive industries, resulting in agricultural companies that instead focus on publicly available or imported technologies. Thus, in contrast to the trends occurring in the U.S. and other developed countries, relatively few researchers in developing countries understand IPR and biosafety and their relationship to biotechnology, and they may also lack experience in dealing with the private sector. Realizing the positive impact of biotechnology will depend to a large extent on the ability of developing countries to access and/or generate technology suitable to their needs. The first question for USAID was how to promote the access of developing countries to new biotechnologies that were appropriate to address local and regional agricultural constraints, but which were found in the private sector (or held as proprietary information by the public sector) in developed countries. The second question was how to ensure that biotechnology was not only an academic research pursuit but that it could be applied in the field in a manner consistent with USAID's goals of sustainability. This raised the importance of biosafety and risk assessment issues, and the development of local regulatory systems along with the capacity to ensure the safety of biotechnology to both human health and the environment.

ABSP at Michigan State University

The Agricultural Biotechnology Support Project (ABSP), funded by the U.S. Agency for International Development (USAID), was established in 1991 as the premier US-sponsored agricultural biotechnology program designed to assist developing countries in accessing and using biotechnology to alleviate local agricultural constraints. For the past 11 years, it has worked in Asia, the Middle East, Latin America and Africa to develop a number of crops with improved agronomic traits. ABSP, managed and implemented through Michigan State University in collaboration with other U.S. universities and the private sector, has integrated research, product development, and policy/regulatory development to assist developing countries in accessing and generating biotechnology and in establishing a regulatory framework for production of biotech crops. In the eleven years of the ABSP, the project has had successful policy, research and product development collaborations at a bilateral level, that is, directed towards individual institutions in specific countries such as Costa Rica, Egypt, Morocco, Indonesia, Kenya, South Africa and India focusing on the application of plant biotechnology to address production constraints in food crops such as banana, cucurbits, maize, pineapple, potato, sweet potato, tomato and mustard oil. ABSP has also been successful in assisting with the development of the appropriate policy framework in developing countries through technical backstopping in biosafety regulatory development and intellectual property rights. A multilateral approach was taken in later years of ABSP in for example the development of the Southern Africa Regional Biosafety (SARB) Program, and in the assistance given to the Association for Strengthening Agricultural Research in East and Central Africa (ASARECA) to develop a strategy for a biotechnology and biosafety program in the region.

At its conception, ABSP represented a novel, integrated approach to agricultural biotechnology research and development programs establishing linkages between developing country public and private sectors and the US private sector, where much of the technology still lies, as well as the US public sector, which has a good history of capacity building and cooperation with industry. Partnerships, both formal and informal, were developed with several private sector companies both in the U.S. and in developing countries, and in addition to applied research and development component, the ABSP project allocated significant resources to address the policy issues that affect the adoption of biotechnology, particularly in the areas of biosafety and intellectual property. The project's use of creative partnerships, between public and private sector laboratories in the US and developing countries, raised many intellectual property (IP) issues as they relate to the contractual obligations of all parties involved. Also, in moving biotechnology out of the laboratory and into the field for testing, the project faced the challenge of building local capacity in biosafety and risk assessment to ensure that the products of the research were deployed in an environmentally safe manner. The development and implementation of favorable policies to govern the important issues of biosafety, food safety, intellectual property rights and technology transfer are essential in order for countries to access these new technologies and commercialize biotechnology products. A sound policy framework is also required if countries are to meet the requirements of international treaties, to facilitate trade and the receiving of food aid, and to attract private sector investment.

ABSP believes that the tools of biotechnology can be applied safely and effectively to address crop production constraints in developing countries. Managed correctly and safely, biotechnology will play an important role in improving food and forage crop production and thus food security in many areas of the world. Although the causes of hunger and poverty are complex, for the majority of people who live in rural areas of developing countries and who are still dependent on farming, low agricultural productivity is still the primary reason why they are poor and hungry. Scientific solutions to improving crop productivity, including the appropriate application of biotechnology, can empower the rural sector by increasing food production, enhancing income for the small farmer, and thereby improving local and national nutritional security.

ABSP Goals

The overall goal of the first Phase of the ABSP Project was:

To mutually enhance U.S. and developing country institutional capacity for the use and management of biotechnology research to develop environmentally compatible, improved germplasm.

Within this overall goal, the objectives were to produce a number of transgenic crops and field-test them in the US and collaborating countries. The project also aimed to develop innovative micropropagation methods and carry out genetic stability tests. Additionally, the training of scientists, administrators, and policy makers on the application of biosafety procedures and intellectual property rights in biotechnology was an important priority. Specific project objectives were as follows:

- ◆ To develop disease-free, high quality planting material of tropical crops, specifically banana, pineapple, coffee, and palm.
- ◆ To assemble vectors containing insect and virus-resistant genes.
- ◆ To genetically engineer potato, sweet potato, and maize for resistance to virus and insect pests in developing countries.
- ◆ To genetically engineer cucurbits with a virus coat protein for development of resistance to potyviruses.
- ◆ To transfer scientific knowledge and techniques to developing countries through postdoctoral fellowships.
- ◆ To demonstrate pest resistance of transgenic crops and integrate this into sustainable agricultural systems via collaborations.

ABSP Phase II

In 1998 as part of a review of the progress of the project, a slightly revised overall goal was developed for Phase II of the project's activities:

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

This goal has been approached by addressing two major objectives:

- i. ***Establishment of a policy framework in developing countries and transition economies that promotes the use, management and commercialization of biotechnology by both host country and multinational agribusiness and research institutions.***
- ii. ***Improvement of marketed crops through strategic research partnerships between the US and developing country public and private sectors.***

Selected Achievements of ABSP

The ABSP project has produced products that will address agricultural constraints of our collaborating countries. It has succeeded in linking together public and private sector institutions, research efforts and policy analysis, and training in intellectual property and biosafety. It has maintained a geographic focus while building a global network to provide information to a wide cross section of scientists, administrators and policy makers in both the developed and developing world.

For example, the ABSP has:

- ◆ Assisted Indonesia in drafting its Plant Variety Protection legislation
- ◆ Assisted Indonesia in establishing an Office of Technology Transfer within the Agency for Agricultural Research and Development
- ◆ Assisted Morocco in adopting and implementing its Plant Variety Protection legislation
- ◆ Assisted Egypt in establishing its Offices of Technology Transfer in the Ministry of Agriculture and the Agricultural Genetic Engineering Research Institute
- ◆ Assisted Egypt, Indonesia and Kenya in developing, adopting and implementing their field-testing guidelines
- ◆ Assisted Kenya in developing sweet potatoes with resistance to Feathery Mottle Virus, recently field-tested in Kenya
- ◆ Developed virus-resistant squashes and melons and field-tested them in Indonesia, Egypt, the Philippines, Jordan, and Brazil
- ◆ Developed potatoes with resistance to potato tuber moth and field-tested them in Egypt and in South Africa
- ◆ Provided educational opportunities to developing country scientists and policy makers in biotechnology, biosafety and intellectual property rights
- ◆ Published a training workbook (*Biosafety & Risk Assessment in Agricultural Biotechnology: A Workbook for Technical Training by Patricia L. Traynor, Robert J. Frederick, and Muffy Koch*) designed to complement technical biosafety-assessment training courses in developing countries
- ◆ Assisted ASARECA with the development of a strategy for a regional Biotechnology and Biosafety program
- ◆ Assisted Southern Africa with a major capacity building program in biosafety through the SARB project

Beyond ABSP- what remains to be done?

While the ABSP has successfully fulfilled its mandate, it has barely begun to address the overall need and interest in the development of human capacity in biotechnology. Although the project has striven to develop a critical mass of trained personnel for the multi-disciplinary teams that have the responsibility for developing and using biotechnology in developing countries, budget constraints have prevented us from training the optimum number of scientists required in individual partner countries and in a regional context.

Additionally, policy constraints, both within developing countries and within export markets, continue to hinder the adoption of the technology. Field-testing has been hampered by a lack of clear biosafety and/or intellectual property guidelines. Currently, the lack of adequate food safety polices, legislation and enforcement are hindering those materials that have been field-tested to move into larger field tests and consumer acceptance studies. The slow adaptation of stronger intellectual property protection has also blocked access to proprietary materials. Finally, the

resistance to genetically engineered products within Europe and other developed countries continues to be a barrier to the adoption of certain crops with export (and therefore significant economic) potential. The costs associated with these additional regulatory constraints are also a deterrent to the use of the technology for many developing country research institutions.

What can development projects such as the ABSP do to overcome these barriers? From our experience, additional capacity building is needed for developing countries interested in accessing and developing agricultural biotechnology. These educational opportunities would not only be in the technical sciences, although this is a critically under-funded area, but would also include business and management training, the development of entrepreneurial and negotiation skills, and continued training on intellectual property as it relates to accessing and using proprietary technologies and biosafety as it relates to implementing field tests, development of food safety laws, and food safety analysis. Continued linkage building between developed country institutions, including the private sector, and developing country research institutions and universities is also critical. The linking of targeted research with policy development and implementation will continue to be crucial to the successful deployment of biotechnology-derived crops.

ABSP believes that successful adoption of biotechnology products in developing countries will only be achieved by approaching it in a comprehensive manner. A cornerstone of this approach is the integration of collaborative research and technology development with activities designed to promote an enabling policy environment for the adoption of biotechnology-derived products. Although this comprehensive system for the development and use of biotechnology is not entirely unique, it presents challenges different from those involved in the adoption of other agricultural technologies. Biotechnology is surrounded by controversial issues, including how to ensure that its use is safe, the dominant role of the multinational private sector, and gaining access to proprietary technologies. There is also a common perception that the public will not ultimately benefit from these technologies. A crucial and often overlooked component of biotechnology programs is the ability to deliver the end products of research to farmers and end users. The development of efficient agribusiness systems for production, distribution, and commercialization of products derived from biotechnology is a key factor in the success of any research program with the goal to take laboratory results to practical application. These are some of the issues that should be taken in taken in consideration in the development of follow-on programs to ABSP.

Lessons learned during ABSP

Many important lessons have been learned during the 12 years of ABSP, and it is impossible to do more than summarize some of them within the scope of this paper. The overall achievements of the project were impressive in terms of both technology and policy development in the developing countries in which the project was involved. It is obvious, however, that the impacts of the project were greatest in those countries in which the program had greater financial resources and hence more and broader activities, specifically in Indonesia and Egypt.

In any assessment of the project it is also important to consider the context of the period in which ABSP was designed. It was, at its inception (and possibly remained so) a unique and highly innovative program, begun at a time when the promise of biotechnology in agriculture was largely a pipe dream, and any practical application, or impact of biotechnology in agriculture, in either the developed or developing world, was still almost decade away. It was not until 1996 that the first transgenic crops were commercialized and since that time the growth in the application of this technology in agriculture has been more rapid than the adoption of any other agricultural technology in history.

Many of the difficulties encountered during the course of the project could not have been anticipated twelve years ago. The global backlash against agricultural biotechnology that was begun and then exacerbated by certain 'green' NGOs and the effect that this would have on issues of international trade and the acceptance of the technology in Europe and in many of the

developing countries greatly affected the development of ABSP as well as countless other similar initiatives.

The ABSP was a bold experiment in forward-thinking by USAID and all the additional partners involved, and as such the outputs and impacts that the program has achieved over 12 years can be considered all the more remarkable. As one indication of the success of this project, many of the experiences of the ABSP and the lessons learned in its lifetime have since been incorporated into the design of many other projects in agricultural biotechnology initiated by the international donor community.

Over its lifetime, ABSP has had success in the following areas:

- ◆ **Innovative linkages:** The project was the first of its kind to forge highly successful private-public, public-public, and private-private linkages. These relationships created enabling environments in developing countries for the application of agricultural biotechnology.
- ◆ **Biotechnology Awareness:** ABSP created positive awareness of biotechnology in developing countries, and particularly highlighted the importance of policy aspects in planning research activities in collaborative projects.
- ◆ **Research Expertise:** ABSP successfully fostered the development of high-quality national biotechnology research expertise in its collaborative projects, particularly in Egypt and Indonesia.
- ◆ **Policy Development:** ABSP provided assistance, training and advice in the methods for biotechnology policy development to a wide range of relevant officials in developing countries. Several countries which received particular policy focus (e.g. Kenya, Egypt, Indonesia, and Morocco) have developed biosafety guidelines and legislation that has permitted at least field-testing of transgenic crops.
- ◆ **Improved crops:** Several improved varieties of crops of relevance and importance to developing countries have been developed e.g. tuber moth resistant potato, virus resistant cucurbits, and virus resistant sweet potato. While none of these have yet reached the point of commercialization, several have undergone field trials and show promise for future development. Given the context and the changing policy environment in which ABSP has operated over the past 12 years, this is an impressive achievement.

Some specific lessons can be pulled out from the many that could be documented, and several of these will be outlined below.

◆ ***Research should be based on local priorities:***

This lesson at first glance seems self-evident, and something that should always be the case in development projects, whether in biotechnology or more 'conventional' agricultural research. Research projects should be based on target country (local) priorities and local decision-making which will during the life of the project serve to foster ownership, encourage commitment, and stimulate the follow-through needed for the ultimate success of the project in terms of product development and commercialization. However, in 1991, at the inception of the ABSP, the project had a mandate that hoped to have transgenic products for field-testing and possible commercialization within six to ten years. Under these circumstances, and given the limited number of transgenic technologies in an equally limited range of crops available, there was no alternative to using the already available, "off-the-shelf" technologies in situations where these could meet an identified need. This approach to some of the early research projects brought about the development of an initial research agenda that was largely "technology-driven", and this led to some of the resulting research products not fitting as well as expected into the target countries' national priorities. In some, though not all cases this led to a number of downstream complications involving IPR and biosafety issues. Overall this approach can also make it more difficult for national decision makers to develop and defend a sense of national ownership of the technologies.

By the later stages of the project this situation had of course changed significantly, the science having evolved so fast that most crops can now be transformed successfully, and gene discovery in a reasonable time frame seems feasible for many situations. In order to maximize the success of collaborative projects, all should be based on a biological assessment of needs and opportunities, and with wise use made of socio-economic, market and other analyses appropriate to a project targeted at ultimate commercialization, including the participation of the appropriate national stakeholders in initial and ongoing priority setting throughout the life of the project.

♦ ***Research-Driven Policy Development***

Establishment of the enabling policy environment for the adoption of biotechnology derived products is critical in developing countries, and should include: an effective biosafety regulatory framework; functional IPR policy and Technology Transfer regimes; and constructive trade policies. Technology development and research collaborations can be the driving force for effective national policy development, as experienced for example in Egypt and in Kenya. In the experience of ABSP, changes in both institutional and national biosafety and IPR policy have been far easier to bring about in an environment where the target country has something to gain, e.g. a transgenic crop with improved pest and disease resistance traits appropriate for the local environment. In such a case there is a large incentive to push both institutional and national policies forward. For this reason, ABSP placed a strong and very effective emphasis on the importance of linking capacity building in biotechnology policy to its applied research projects.

Thus in Kenya it was possibly the availability of virus resistant sweet potatoes (developed by the local public sector in collaboration with Monsanto), and in Egypt the tuber moth resistant potatoes (developed by MSU and AGERI) that accelerated the development of biosafety frameworks in those countries. It is our assertion that other donor programs that have sought to build capacity in biosafety policy have had less success than ABSP because they have been trying to build capacity 'in a vacuum' in which the target audience can see no immediate need for development and implementation of the policy.

♦ ***IP and biosafety considerations should be addressed at the outset of research projects***

Issues of Intellectual Property were recognized to be important during the initial phases of ABSP and were therefore brought into the various research projects at the very early stages. However, even with such a forward-looking and enlightened approach, this did not prove to be a simple process. Several factors affected this and have continued to do so over the life of the project. For example, in the last several years the dynamic nature of the corporate takeovers within the life sciences field has been a major complicating factor. The mergers of several of the companies with which we were negotiating over specific pieces of IP made matters very confusing. At times even the companies themselves were not sure of who owned the specific primers and constructs that were used in the research projects, and in some instances these issues are still to be resolved pending patent decisions. In some instances this negotiation hinged on interactions with one individual within the company, and when that individual left the company negotiations had to begin from scratch. This can be an intensely frustrating and demoralizing experience. Many companies have changed their stance considerably in terms of the arrangements for the use of their IP in recent years, and this situation is still very fluid as there is now a greater awareness by some companies of the issues facing developing countries, and the public good that could be done by donating or licensing technologies under reasonable terms for use in crops of importance to developing countries and unlikely to have a lucrative export market. Twelve years ago this was largely an unknown area, and companies were perhaps more wary than today to allow their IP to be released to the public sector.

Obviously in addition to a forward-thinking approach to IP issues, biosafety regulatory

considerations should also be included at the beginning of any project, and where possible, moves made towards the development of regulatory packages early in the product development phase. These can take a very long time to complete, particularly where considerable food safety issues are involved. The financial input required to put potential products through such detailed testing is also considerable, and would be out of reach of many public sector-funded projects. This emphasizes the importance of the involvement of the private sector where possible in taking forward the products of research projects out of the laboratory and field test stage towards commercialization.

♦ ***Public-Private sector partnerships are critical.***

Although often challenging and complex, involving the private sector in research projects from the very early stages is vital if developed products are ever to reach farmers' fields. This is obviously important due to the IP and biosafety considerations summarized above, and also due to the fact that for many crops the involvement of the private sector is essential in the dissemination of resulting products. One of the biggest roadblocks in any agricultural development projects lies in getting improved crops out of the laboratory and into the hands of farmers. This is not a unique problem to biotechnology, although some of the considerations may be different for transgenic crops. In simplest terms, involvement of the private sector in dissemination may involve, for example the local seed sector or local tissue culture companies in scaling up and/or distributing improved seed or tissue culture plantlets to farmers. Ideally however, the private sector should be involved in the earlier stages to ensure their participation and ownership of the project.

ABSP Indicators and Achievements 1991 – 2003

ABSP Indicators and Achievements 1991–2003

This section compares the anticipated project outputs linked to objectively verifiable indicators, as defined by USAID in the ABSP Project Paper (April 1991), to actual results and impacts of the ABSP/MSU program. These indicators, part of the original project paper of the ABSP developed by USAID, were established in 1991 and provide measurable outputs for success of the project. While the project's goal and objectives were revised slightly in 1997, the indicators were designed for a 10-year project and are therefore still valid measures for the success of the ABSP.

In 1998 as part of a review of the progress of the project, a slightly revised overall goal, objectives and indicators were developed for Phase II of the project's activities. These indicators overlapped considerably with the indicators developed for Phase I of the project, and for the purpose of this final report they will be merged under the general headings below where possible. Where they differ, this will be indicated in the text.

Priority Research and Technology Transfer

◆ Develop multi-disciplinary applications of biotechnology for tropical crop improvement

Indicators: Three recombinant and/or tissue culture technologies developed for tropical crops

- ◆ New bioreactor micropropagation methods developed for banana and pineapple.
- ◆ Improved tissue culture techniques for banana.
- ◆ Transgenic cucurbits with resistance to Zucchini Yellow Mosaic Virus.
- ◆ Transgenic maize with resistance to Asian Corn Borer.
- ◆ Transgenic tomatoes with resistance to geminivirus.
- ◆ Transgenic potatoes with resistance to Potato Tuber Moth
- ◆ Transgenic sweet potato with resistance to Sweet Potato Feathery Mottle Virus
- ◆ Improved transformation methodologies for cucurbit species.
- ◆ Protocols for transformation of mustard with genes encoding β -carotene.

◆ Develop publications and/or patents as documentation of technical accomplishments

Indicators: Three patents; six publications

- ◆ Publication of more than 100 peer-reviewed papers as well as numerous review articles, book chapters, abstracts, posters and presentations at national and international meetings (see full publication list).
- ◆ Filing of three patents.
 - Development of a method for the *in vitro* sexual reproduction of corn plants (MSU)
 - Development of a method for asexual *in vitro* propagation of corn plants (MSU)
 - Isolation and characterization of maize specific promoters (Pioneer Hi-Bred (*WIPO PCT WO 00/20571*, 13 April 2000))

◆ **Small-scale field tests of biotechnology-derived plants conducted in the U.S. and LDCs to establish agronomic acceptability of germplasm**

Indicators: Nine field tests of biotechnology-derived plants conducted in the U.S. and LDCs

- ◆ Three transgenic maize lines selected for continued research in the Midwest region of the US (ICI/Syngenta).
 - ◆ 2 lines: *A188xBE73* and *NP19*, each transformed with *Cry1A (c)* and *Cry1V* genes from *Bacillus thuringiensis* for resistance to Asian Corn Borer and field tested in the US.
 - ◆ Several lines of transgenic potatoes with resistance to potato tuber moth field-tested in Michigan (7+ years).
 - ◆ *Spunta G2* and *Spunta G3*, from a locally grown Egyptian variety transformed with a *CryV* vector.
 - ◆ *Lemhi Russet* transformed with *Cry1A(c)* and *CryV*
 - ◆ *Atlantic* transformed with *CryV*
 - ◆ Several lines of transgenic potatoes with resistance to potato tuber moth field-tested in Egypt (5+ years, 3 locations).
 - ◆ Transgenic potatoes with resistance to potato tuber moth greenhouse and field tested in South Africa (2 years, 2 locations).
 - ◆ Transgenic virus resistant cucurbits field-tested in Egypt (AGERI). (1999-2003)
 - ◆ *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*
 - ◆ *Eskandarani squash* transformed with the coat protein gene of *ZYMV* greenhouse and field-tested in Egypt.
 - ◆ Transgenic virus resistant tomato field-tested in Egypt (AGERI). (2000-2003)
 - ◆ *Genotype UC82B* engineered for resistance to geminiviruses using the coat protein, the pre-coat protein, the replicase gene or the *AV4* gene of Egyptian geminiviruses.
 - ◆ Virus resistant sweet potato field tested in Kenya (KARI 2001-present)
 - ◆ *African variety, CPT-560* transformed with the coat protein gene of the sweet potato feathery mottle virus (*SPFMV*) and field-tested in Kenya (2001-present).
- ◆ **Registration and distribution of improved germplasm proven to be field-tolerant for adverse pests, pathogens, and environments**

Indicators: Twelve improved genotypes more tolerant of adverse environments, pests, and pathogens distributed

- ◆ **Potatoes:** Many lines have been shown to have resistance to potato tuber moth in the field (see above). However these have not yet reached the stage of variety registration or distribution to farmers.
- ◆ **Cucurbits:** Several transgenic lines with virus resistance have been field tested, but none so far have been registered or distributed to farmers. However, many conventionally-bred lines have widely distributed from the breeding program at Cornell:

- ***Cucurbita pepo*** -- with resistance to one or more viruses. Lines field-tested in Egypt, Jordan, South Africa, the Philippines, Indonesia, and Brazil.
 - ***Cucurbita moschata*** -- virus resistance has been crossed into tropical types and plants field tested in the Philippines. Butternut types with multivirus and powdery mildew resistance field-tested in South Africa.
 - ***Cucumis melo*** – resistance to viruses and powdery mildew in sweet orange flesh netted shipper types and sweet crisp green and white flesh melons.
 - ***Cucumber*** -- Breeding lines created in Beit and Asian 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. Field tested in Egypt, the Philippines, Indonesia, S. Africa, and Brazil.
- ◆ **Marketing analysis, feasibility study, biosafety regulatory status in LDCs; economic impact analysis**
- Indicators: One analysis, one feasibility study, one biosafety study; conduct two economic impact analysis for project outputs**
- ◆ Socioeconomic impact analysis conducted for the potential impact of micropropagation technologies developed with Agribiotecnologia de Costa Rica (Costa Rica), Fitotek Unggul (Indonesia), and DNA Plant Technologies (CA).
 - ◆ A three-year project assessment of AGERI, Egypt--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 and produced 3 reports.
 - *Commercialization Prospects for AGERI (1999)*
 - *Strategic Marketing Plan for AGERI (2000)*
 - *Preparing AGERI for Continued Success in the Evolving Biotechnology Industry, (2001)*
 - ◆ Analysis of biosafety legislation, regulation, field-testing and commercialization in Africa conducted by Innovation Biotechnology in collaboration with ISNAR.
 - ◆ A white paper developed for USAID on Biosafety Issues for Sub-Saharan Africa.
 - ◆ *Ex Ante* assessment of new cucurbit seed varieties in Indonesia and South Africa (2001)
 - ◆ Economic analysis of genetically modified potatoes in South Africa and Egypt (2001)
 - ◆ Grades & Standards Assessments--four assessments carried out on the need for and importance of grades and standards (G&S) for select commodities Kenya, Malawi, Mozambique and Zambia. (2000-2001)
 - ◆ USAID Biotechnology Program Development in Africa, ABSP managed Biotechnology Assessments in South Africa, Uganda and Kenya. (2001-2002)
 - ◆ Background paper on biosafety prepared for ASARECA—*Regulatory Status And Regional Biosafety Regulatory Mechanism And Administration Under ASARECA*. Ms Muffy Koch Innovation Biotechnologies, South Africa. 2001

- ◆ Background paper to assist the ASARECA Working Group in identifying priority opportunities for research, adaptation of existing technology, and technology transfer—*Agricultural Biotechnology in the ASARECA Region: Priorities for Research*. Dr. Andrea Johanson, ABSP, Michigan State University. 2001

Innovative Activities

- ◆ **Establish commercial links for appropriate technologies developed between U.S. companies and universities and LDC institutions**

Indicators: Two commercial and managerial links developed among U.S. companies and LDC institutions, and three industry-based management seminars

- ◆ ABSP conducted one industry-based management seminar in April 1993.
 - ◆ Private-private linkage supported between DNA Plant Technology (Oakland, California), Agribiotecnologia de Costa Rica (ACR) and Fitotek Unggul (Jakarta, Indonesia).
 - ◆ Private-public linkage support between ICI Seeds (now, Syngenta, Slater, Iowa) and the Central Research Institute for Food Crops (Bogor, Indonesia).
 - ◆ Private-public linkage supported, in part, between the Kenya Agricultural Research Institute (KARI) and Monsanto Company (St. Louis, Missouri).
 - ◆ Private-public linkage supported between Pioneer Hi-Bred (Johnston, Iowa) and the Agricultural Genetic Engineering Research Institute (Egypt).
 - ◆ Private-public linkage between Monsanto Company (St. Louis, Missouri) and TERI (India).
 - ◆ An International Symposium on “*Biotechnology for Food & Nutritional Security*” was organized by TERI in December 2002, bringing together private and public sector partners in the project.
 - ◆ CUB Symposium—a joint effort of AGERI and ABSP, funded by USAID/ATUT, held May 2000 to bring together public and private sector partners in the projects in Egypt
- ◆ **Increased awareness of LDC officials through internships in IPR and in development of regulatory policy through positions in the U.S. regulatory agencies**

Indicators: Six internships provided for (a) protection of IPR in U.S. legal firms (4); and (b) scientifically-based biosafety regulatory policy in U.S. regulatory agencies (4)

- ◆ *IPR and Patent Internship Program* designed and implemented by ABSP with Professor John Barton of Stanford Law School. Seven interns from Egypt, Kenya and Indonesia participated. (1993)
- ◆ *Workshop on Intellectual Property Rights, Patents & Licensing, Cairo, Egypt* organized by ABSP with Prof. John Barton. Attended by over 100 participants from various public and private sector institutions. (1994)
- ◆ *IPR Workshop*, Washington DC from. Forty-four participants attended from Egypt, Kenya, Indonesia and Costa Rica, Thailand, Sri Lanka. (1994)
- ◆ Eight week *Biosafety Internship Program* to assist collaborating countries in the development of biosafety guidelines. Attended by seven scientists from Egypt, Kenya and Indonesia. The Indonesian scientists then received training at ICI Seeds, Iowa, and the Egyptian and Kenyan Scientists received

training at Michigan State University. The group interacted with federal personnel at USDA/APHIS, FDA and EPA responsible for biosafety and the laws and regulations governing the introduction of GMOs. (1993)

- ◆ *Latin America & Caribbean Region Biosafety Workshop*, Jamaica. Organized by ABSP in collaboration with the Bean/Cowpea CRSP. Attended by 42 representatives from 12 countries. (1993)
- ◆ *Biosafety Workshop*, AGERI, Egypt, to create a greater awareness and strengthen the biosafety regulatory framework in Egypt and the Middle East. Involved international experts on in biosafety, scientists and regulatory personnel from Egypt and Africa. (1994)
- ◆ *Biosafety Internship Program: Risk Assessment and Risk Management*. Organized by ABSP and the Institute of International Agriculture at MSU in cooperation with the Information Systems for Biotechnology Program at the Virginia Polytechnic Institute (VPI). A 2-week internship program, attended by 15 international participants. (1996)
- ◆ *Plant Variety Protection and Patents Workshop*, Indonesia. Fifty senior representatives of the government and private sector in Indonesia attended. (1996)
- ◆ *Internship program in IPR and Technology Transfer*, at MSU organized by ABSP, with the Office of Intellectual Property and the Institute of International Agriculture. (1996)
- ◆ *IPR Internship*, MSU. The success of the above internship program led MSU to develop it into a short course that has now been offered annually since 1996. During the last five years, 84 international participants have attended this program. (1996-2003)
- ◆ *Colloquium on Plant Variety Protection and Patents*, Rabat Morocco. To assist Morocco in the implementation and enforcement of a new PVP law pending approval from the Moroccan parliament. Over 250 representatives from governmental agencies and the agribusiness community attended. (1997)
- ◆ ABSP provided three Moroccans from the DPVCTRF (the organization charged with implementing the Plant Variety Protection law) with additional training at the Plant Variety Protection Office of USDA in Washington DC. ABSP also provided logistical support to purchase computer equipment and software to outfit the new PVP office within the DPVCTRF. (1998)
- ◆ *Workshop on The Impact of Intellectual Property Rights on International Trade and Agriculture in East Africa*, Kampala, Uganda. 70 participants attended the workshop from Ethiopia, Kenya, Nigeria, Tanzania, Uganda, Zambia, Zimbabwe, Switzerland, United Kingdom, France, United States, Costa Rica, South Africa, and the Netherlands. (1999)
- ◆ *Southern Africa Regional Biosafety (SARB) Program Workshops and other meetings related to sub-regional biosafety needs and opportunities*.
 - ◆ More than 37 individual workshops held in the region.
 - ◆ More than 600 people sensitised to biosafety issues.
 - ◆ 380 scientists trained in the basics of conducting a risk assessment of GM crops or products.

◆ Co-ordination with other USAID projects

Indicators: Co-sponsor one international conference with one USAID and/or CRSP, one CRSP and one natural resource representative appointed

to the TAG, one USAID mission requested technical/institutional TDY support

- ◆ ABSP jointly sponsored a Regional Latin America Biosafety Workshop in Jamaica with the Bean/Cowpea CRSP (1993).
 - ◆ Dr. Ronald Carroll of the University of Georgia and a member of the Sustainable Agriculture and Natural Resource (SANREM) CRSP, served on ABSP's first Technical Advisory Group (TAG).
 - ◆ ABSP sought the expertise of the International Agricultural Research Centers (IARCs) by selecting Dr. Mujeeb Kazi from CIMMYT and Dr. Gurdev Khush from IRRI to be members of the TAG.
 - ◆ Intellectual property rights policy assistance to the Bean/Cowpea CRSP--the USAID-supported Bean/Cowpea Collaborative Research Support Project (CRSP) developed an IP policy with assistance and guidance from ABSP--the first time a CRSP project has institutionalized an IP policy with their U.S. and overseas collaborators. (2000)
 - ◆ Grades & Standards Assessments--four assessments carried out on the need for and importance of grades and standards (G&S) for select commodities Kenya, Malawi, Mozambique and Zambia. (2000-2001)
 - ◆ USAID Biotechnology Program Development in Africa, ABSP managed Biotechnology Assessments in South Africa, Uganda and Kenya. (2001-2002)
 - ◆ Additional Support from USAID or other donors includes:
 - ◆ USAID/Jakarta (Indonesia): \$1.6 million
 - ◆ USAID/Egypt: \$7.0 million
 - ◆ USAID/Africa Bureau: \$400,000
 - ◆ USAID/Rabat (Morocco): \$200,000
 - ◆ REDSO: \$300,000
 - ◆ Rockefeller Foundation: \$15,000
 - ◆ Monsanto Company: \$2,000
 - ◆ Garst Seed Company: \$2,000
- ◆ **Strengthen ties among LDC institutions and private institutions, and commercial biotechnology trade association**
- Indicator: Co-sponsorship of seven memberships in a biotechnology trade association**
- ◆ ABSP's developing country partners received full memberships in the Biotechnology Industry Organization (BIO) for the duration of their involvement in the project. This includes Scripps Research Institute, KARI, CRIFC, Fitotek Unggul, ACR, and AGERI.
- ◆ **Provide legal expertise to project's institutions**
- Indicators: Two USAID provided technical support, IPR, biosafety internships or consulting, network activities; write and file three patent applications from project research and/or license**
- ◆ ABSP has, when needed, procured the legal expertise of MSU's Office of Intellectual Property and MSU's patent attorney.
 - ◆ Professor John Barton, acting as an IPR consultant to ABSP, has actively assisted several developing countries in designing and implementing IPR legislation.

- ◆ A two-week internship program in IPR and technology transfer was organized at MSU in February 1996. This was organized by ABSP in cooperation with the Office of Intellectual Property and the Institute of International Agriculture at MSU.
 - ◆ The success of ABSP's first internship program led MSU to develop it into a short course that has now been offered annually since 1996. During the last eight years, more than 125 international participants from 25 countries attended this program. The ABSP project has directly sponsored participants from the following seven countries: Colombia (1), Egypt (3), Ethiopia (1), India (3), Indonesia (5), Philippines (1) and South Africa (2)
 - ◆ ABSP provided three Moroccans from the DPVCTRF (the organization charged with implementing the Plant Variety Protection law) with additional training at the Plant Variety Protection Office of USDA in Washington DC. Following this training, the ABSP provided logistical support to purchase computer equipment and software to outfit the new PVP office within the DPVCTRF.
 - ◆ ABSP has supported the filing of two patents (U.S. 5,281,529 and U.S. 5,320,961).
 - ◆ ABSP developed a manual for biosafety training: *Biosafety & Risk Assessment in Agricultural Biotechnology: A Workbook for Technical Training*. Patricia L. Traynor, Robert J. Frederick, Muffy Koch (ISBN: 1-56525-016-8).
 - ◆ ABSP's Intellectual Property Consultant, Dr. Fred Erbisch, authored a training manual based on the IP and Technology Transfer Course at MSU: *Basic Workbook In Intellectual Property Management*. Available online November 2003
- ◆ **Environmental analysis, field testing and germplasm distribution**
- Indicator: Prepare three reports for environmental and field testing protocols***
- ◆ ABSP has prepared reports for USAID's Biosafety Committee for the field-testing of transgenic potatoes in Egypt. A similar report has been prepared for field-testing in Indonesia, and South Africa.
 - ◆ ABSP conducted an evaluation of natural enemy populations of potato tuber moth, coupled to an evaluation of plant host preferences for PTM in Egypt. (2001)
 - ◆ Three papers were published by Grumet *et al.* in 1997 on i) the opportunity for escape of engineered genes from transgenic crops; ii) the effect of border rows on pollen mediated gene movement, and iii) a direct comparison of pollen mediated movement of native and engineered genes. (1997)
 - ◆ Sorghum gene flow case study—Although no suitable GMO sorghum could be located, 2 field trials were designed incorporating conventional seed to measure geneflow. These were conducted by the ARC in South Africa, and NUST/ICRISAT in Zimbabwe. (2002)

Networking Activities

- ◆ **Provide support to three LDC tissue culture facilities to enhance their technical capabilities, increase efficiency in biotechnology, integrate research with conventional plant breeding, and service as facilitators**

- ◆ Supported commercial tissue culture facilities in Indonesia and Costa Rica (Fitotek Unggul and ACR).
- ◆ Provided technical assistance to CRIFC, KARI, Kenyatta University (Kenya), AGERI, and the National Research System of Uganda to train personnel to carry out improved tissue culture technology.
- ◆ Supported participation of five Africans from Kenya and Uganda in UNESCO tissue culture course in South Africa.
- ◆ Provided limited funds to developing country partner institutions (KARI, CRIFC and ACR) for needed equipment and supplies to continue to develop tissue culture and molecular techniques.

◆ **Convene international and regional or country-specific conferences**

Indicators: One international conference to include workshop on research management, three regional or country-specific conferences

The project has held or co-hosted the following workshops:

- ◆ *Latin America/Caribbean Regional Biosafety Workshop*, May 1993, Jamaica.
- ◆ *Biosafety/IPR/Project Review Workshop*, January 1994, Egypt.
- ◆ *IPR Workshop*, July 1994, Washington, DC.
- ◆ *Mediterranean Regional Genetic Diversity Workshop*, June 1994, Egypt.
- ◆ *ABSP/Indonesia Technology Transfer Workshop*, April 1995, Indonesia.
- ◆ *Indonesia IPR Workshop*, March 1996, Indonesia.
- ◆ *International Biotechnology for a Better World Conference*, April 1997, California.
- ◆ *Workshop on The Impact of Intellectual Property Rights on International Trade and Agriculture in East Africa*; January 1999, Uganda.
- ◆ *Southern Africa Regional Biosafety (SARB) Program Workshops and other meetings related to sub-regional biosafety needs and opportunities.*
 - ◆ More than 37 individual workshops held in the region.
 - ◆ More than 600 people sensitised to biosafety issues.
 - ◆ 380 scientists trained in the basics of conducting a risk assessment of GM crops or products.
- ◆ ABSP was a co-sponsor for the Bread for the World Institute (BFWI) Conference on *Agricultural Biotechnology - Can it help reduce Hunger in Africa*, held in Washington DC from March 5-7, 2002. Organized by BFWI through a grant from the Rockefeller Foundation and with support from ABSP. ABSP funding covered the travel and expenses of 10 African delegates. Over 100 participants attended from local and international NGOs. (2002)
- ◆ ABSP co-sponsored a plant biotechnology symposium *Perspectives from Developing Countries: Towards a Global Strategy, Partnership and Action Plan for Food Security and Poverty Alleviation* organized by the Food and Agriculture Organization (FAO) and the Crop Science Society of America. This symposium was held during the Annual Meeting of the American Society of Agronomy, the Soil Science Society of America and the Crop Science Society of America in Indianapolis, Indiana (12-14 November 2002). ABSP funding covered the travel and expenses of 20 developing country participants. (2002)

◆ **Follow-on support provided to trained scientists and their institutions**

Indicators: Three scientists provided follow-up support

ABSP provided institutional support to:

- ◆ *Kenyan Agricultural Research Institute*: Assistance to upgrade tissue culture facilities and conduct mock field trials within Kenya
- ◆ *Central Research Institute for Food Crops (Indonesia)*: Assistance to upgrade laboratories and develop plans for greenhouse. Also assistance via a small grants program to individual researchers.
- ◆ *Research Institute for Vegetables (Indonesia)*: Assistance to construct screenhouse and trial plots
- ◆ *Agribiotechnologia de Costa Rica*: Assistance to improve tissue culture facility and provide support to analyze tissue-culture derived pineapples and bananas.
- ◆ *VOPI, South Africa*: Assistance given by MSU potato researchers in setting up and management of glasshouse and field trials of transgenic material.
- ◆ *AGERI, Egypt*: Assistance given in laboratory, glasshouse and field methods for developing and testing transgenic plants (maize, cucurbits, potatoes and tomatoes). Assistance in design and implementation of a glasshouse containment facility at AGERI.
- ◆ *TERI, India*: Assistance provided by Monsanto in the development of transformation techniques for mustard with genes conferring increased β -carotene.

◆ **Provide biosafety guidance to LDCs**

Indicator: Three biosafety guidances provided

Biosafety guidance has been provided to several of ABSP's collaborating countries on many different levels, including:

- ◆ **One-to-one consultations**
 - ◆ Indonesia, Egypt, Kenya, Philipinnes etc.
- ◆ **In-country workshops**
 - ◆ (see Networking Activities)
- ◆ **Sponsorship to attend other workshops**
 - ◆ (see Capacity Building and Network Activities)
- ◆ **Internship training**
 - ◆ (See Capacity Building)
- ◆ **Support for sub-regional activities**
 - ◆ Activities under SARB
 - ◆ Assistance in biosafety/biotechnology to ASARECA
- ◆ Publication of the ABSP Biosafety Training Workbook used in workshops and training courses as part of SARB and other training initiatives. Over 250 print copies have been distributed to individuals world-wide; and another 350 used in training courses. In addition, the full text is freely available for download from http://www.ija.msu.edu/absp/biosafety_workbook.html, and this web page has been accessed over 2,000 times since January 2003.

◆ **Provide support to developing publications from research conducted at three laboratories**

Indicator: Three publications developed

(See full Publications List)

Joint publications include:

S. Assem, H. Zhong, M.B. Sticklen. 1994. Efficient and genotype-independent regeneration of Egyptian maize.

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.

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 (*Phthorimaea operculella*). *Am. Potato J.*

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.

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.

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., 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.

(See full Publications List on Page88*)

◆ **Provide support to CGIAR centers for regional coordination**

Indicator: Three CGIAR centers identified and supported for regional network activities

Due to budget constraints financial support to CGIAR centers could not be maintained under ABSP.

- ◆ ABSP's Technical Advisory Group under Phase I included two members from the International Centers (Dr. Gurdev Khush at IRRI and Dr. Mujeeb Kazi at CIMMYT).
- ◆ ABSP has sought collaboration with CGIAR Centers regarding the testing of transgenic material (e.g. CIP, Peru and CIP Egypt for testing tuber moth resistant potatoes) and through collaborations with our developing country institutions (e.g. KARI/CIMMYT collaboration on maize).

Capacity Building

◆ Post-doctoral and graduate degree level training in molecular biology and biotechnology in either public or private sector research facilities

Indicators: Six post-doctoral candidates trained, and two graduate degree level scientists trained

- ◆ One graduate student, Dr. Duncan Kirubi from KARI, Kenya, was funded by ABSP and received his Ph.D. from Texas A&M University in 1996. He has since returned to Kenya to continue work on maize transformation. (ABSP did not focus on further higher degree training due to insecurity of funding.)
- ◆ ABSP supported technical training for more than 10 post-doctoral researchers for periods ranging from three months to two years. Researchers have received training at: MSU, University of Wyoming, University of Texas at Dallas, Ohio State University, Cornell University, Pioneer Hi-Bred, and ICI Seeds (Syngenta).
- ◆ ABSP has supported many developing country scientists, administrators, and policymakers for non-degree training in biotechnology policy through participation in short-courses, short-term training, workshops, and internships.

◆ Research to appraise use of biotechnology in the U.S. as related to applications in LDCs

Indicators: Three U.S. biotechnologies appraised for applications in LDCs

The three US biotechnologies that were appraised for application in LDCs were:

- ◆ Bioreactor micropropagation technology for tropical crops (banana, pineapple, coffee and palm);
- ◆ Viral coat protein technology for the development of transgenic virus resistant cucurbits, sweet potato and tomato;
- ◆ Use of Bt toxin genes for transgenic resistance to specific insect pests of maize and potato.

◆ Establishment of a policy framework in developing countries and transition economies that promotes the use, management and commercialization of biotechnology by both host country and multinational agribusiness and research institutions. (ABSP Phase II Objective)

Indicator 1: Science-based policies for IPR, biosafety and/or novel foods drafted and adopted at national and/or regional levels.

- ◆ Indonesian biosafety guidelines developed and approved.
- ◆ Indonesian food safety guidelines for GMOs developed and approved.
- ◆ Egyptian biosafety guidelines developed and approved.
- ◆ Kenya National Biosafety Committee established and guidelines developed and approved.
- ◆ Indonesian PVP Law passed.
- ◆ Technology Transfer office (KIAT) established within the Indonesian Agency for Agricultural Research and Development (AARD).

- ◆ Office of Technology Transfer and Intellectual Property (OTTIP) at AGERI, Egypt established.
- ◆ Adoption of a technology transfer policy within the Egyptian Ministry of Agriculture (ARC).
- ◆ Moroccan Plant Variety Protection Law passed.
- ◆ Plant Breeders' Rights Office established in Kenya.

Indicator 2: Applications for field-testing for improved and/or genetically engineered varieties handled effectively by host country regulatory bodies.

- ◆ **Indonesia:** The Indonesian Biosafety Committee has approved several transgenic crops for contained field trials, including: Bt cotton, Bt corn, Roundup Ready cotton, Roundup Ready corn, and Roundup Ready soybean. Bt cotton is the only crop so far in commercial production in the country.
- ◆ **Egypt:** The Egyptian Biosafety Committee has approved field-testing of several transgenic crops, including insect-resistant potatoes, virus resistant squash, virus resistant tomatoes, Bt cotton and Bt maize. No approvals have yet been given for commercial release of any crop.
- ◆ **Kenya:** The Kenyan Biosafety Committee has approved field trials of virus resistant sweet potato, and several lines of Bt maize and Bt cotton.

ABSP PROJECT SUMMARIES

ABSP Project Summaries

Under the first phase of ABSP, from 1991 to 1998, research was conducted on a limited number of crops chosen either for their importance in food security (sweet potato, potato, maize, banana) or for their importance as a source of economic development (potato, cucurbits, banana, pineapple, and tomato). The research program was focused on assisting developing country institutions to adapt applications of biotechnology to address specific productivity constraints. Research problems identified for inclusion in ABSP Phase I were in two major categories: host plant resistance and micropropagation.

The crops chosen for research were based on economic and nutritional significance coupled with severe pest or pathogen constraints on productivity. The project collaborations involved creative partnerships between both public and private sectors in the US and in the developing countries.

It should be noted that financial support for the various research and policy efforts arose from different USAID offices and programs, influencing the type of duration of research projects. For example, financial support from USAID/Jakarta supported research on the development of maize with Asian Corn Borer resistance, a collaboration between the Central Research Institute for Crops and Garst Seed Company, and additional support for the micropropagation of tropical crops with DNA Plan Technology and Fitotek Unggul. While welcome, the research support from USAID country offices is rarely long-term and rarely lasts through the entire development process. An exception to this rule is USAID/Cairo's strong support for applied research partnerships. In truth, USAID/Cairo has, for several years, provided the bulk of the research support to the ABSP, exerting considerable influence over the types of research projects selected

The second phase of ABSP, which began in 1998, had as its second major objective '*to improve marketed crops through strategic research partnerships between the US and developing country public and private sectors*'. Substantial progress had been made by many of the research projects under ABSP Phase I, and some of these continued under other funding. However, due to budget constraints under Phase II only two of the original projects were taken forward under ABSP Core funding. These were: *Potato transformation for development of tuber moth resistance*; and *Development of virus resistant cucurbits*. The primary reason for selection of these projects for further support was that both of these projects had reached the stage at which products would be ready for field-testing and possibly even commercialization within 3 years. Potato is one of the most important crops worldwide, both in terms of food security and in the development of added-value products, and cucurbits represent a variety of high value crops that play important roles in local diets and in export markets in many countries. It was hoped that these projects would serve as case studies for the transfer of biotechnology-derived products to developing countries.

In the experience of ABSP Phase I, changes in national biosafety and IPR policy are far easier to bring about in an environment where the target country has something to gain e.g. a transgenic crop with improved pest and disease resistance traits. In such a case there is a large incentive to push policies forward. For this reason, ABSP has continued to place a strong emphasis on the importance of linking policy work to applied research projects.

Micropropagation of tropical crops for commercial planting (Costa Rica)

DNA Plant Technology (DNAP), USA
Agribiotecnología de Costa Rica (ACR), Costa Rica

1991-1997

Project Goal

To implement more efficient methods of micropropagation of banana, pineapple, coffee, and ornamental palm for high-quality commercial planting stock. Banana, and pineapple are increasingly important export crops for Costa Rica and Central America, and tissue culture methods help to eliminate problems due to insects, nematodes and some fungal diseases. However, tissue culture is expensive and conventional techniques are not able to meet the high demand for plants. The goal of this project was therefore to investigate the potential of embryo regeneration using bioreactors as an efficient, low cost way to increase production of pineapple and banana.

Project Activities

ABSP's initial private sector collaborator was DNA Plant Technology, a plant biotechnology company in Oakland, California, which came to the project with a host country partner in place-- ACR in Costa Rica, a micropropagation company with laboratory and farm facilities in Costa Rica and an export business in tropical crops and ornamentals. Later, DNAP also had an agreement with Fitotek Unggul in Indonesia (see next section).

The agreement between ACR and DNAP was initiated in 1992 with the general goal of implementing more efficient methods of micropropagation of banana, pineapple, coffee, and ornamental palm for high-quality commercial planting stock. ACR was to provide the germplasm of all the target crops. DNAP was to develop the micropropagation technologies, transfer to ACR the processes and material for field trials, and commercialize the final product. The agreement stipulated that technical training be provided for ACR staff. ABSP provided seed money to support the project.

Project Impacts

Although the project was disrupted by some ownership, location, and personnel changes at DNAP and a decrease in ABSP funding of the project midstream, this private-to-private linkage had a number of positive outcomes:

- ◆ ACR now has a practical pineapple micropropagation system and is following some promising lines in banana regeneration. The collaboration with the ABSP project improved ACR's efficiency in producing banana and pineapple planting materials.
- ◆ Preliminary results for somatic-embryogenesis-derived pineapple plants indicated a much higher than usual rate of variation in plant and fruit size, and other factors.
- ◆ The technology was not successful for banana due to problems with replicating published research and with bacterial contamination. Both DNAP and ACR have continued with banana research and have been optimistic about future success.

- ◆ DNAP provided technical training to ACR staff in the use of short-term bioreactor technology and transformation technology. Three senior scientists from DNAP spent time at SCR providing technical training. Both sides felt that this was very much a co-learning experience.
- ◆ ACR scientists, through the training component of the agreements, became important participants in their countries' biosafety and plant protection policy efforts. Professional liaison and mutual respect established among scientists at DNAP and ACR have resulted in future informal cooperative efforts.
- ◆ The techniques for coffee micropropagation was developed in the early stages of the project and with ABSP funding several hundred plants were later transferred to ACR in Costa Rica for testing. However, organizational changes at DNAP necessitated the discontinuation of this part of the project. However, ACR continued field-testing of plants from earlier work.
- ◆ A small exploratory project with palms was carried out, and of 4 species of palm, peach palm was found to be the most amenable to tissue culture techniques. After budget cuts in early 1994 DNAP work on palm was discontinued. DNAP provided ACR with a zygotic embryo propagation method for peach palm, but this proved to be unfeasible due to the lack of cross pollination in peach palm.
- ◆ Through ABSP's website we continue to pass enquiries on micropropagation and the availability of micropropagated crops to ACR.

This project was successful because DNAP and ACR had a common private-sector culture that included a level of trust and a similar understanding of contract law and confidentiality. The final project assessment report points out that the venture was also successful because it supported co-learning rather than one-way technology transfer. Staff at ACR report that the work they have carried out with ABSP's involvement has enhanced the reputation of their company.

Micropropagation of tropical crops for commercial planting (Indonesia)

Fitotek Unggul, Indonesia
DNA Plant Technology (DNAP), USA

1991-1996

Project Goal

The impetus for this project came from Fitotek Unggul, a small Indonesian tissue culture company because they were receiving large orders for pineapple plantlets for Indonesian plantations. Conventional production of pineapple, although less costly than other means, could not keep pace with the demand, and tissue culture offered a cost-effective method to provide disease-free seedlings year around. The primary goal of this project was to develop methods for pineapple micropropagation in liquid cultures, to transfer the technology to Fitotek, and to begin the process of commercialization.

Project Activities

This collaborative project capitalized on initial development of bioreactor technology for axillary shoot bud multiplication of pineapple in a liquid culture system. It was hoped that this method would be Fitotek's answer for further growth in anticipation of a demand for more than 15 million plants per year. DNAP had extensive experience in tissue culture/regeneration in a large number of plant species, including major tropical plantation crops, and Fitotek had been in the business of plant propagation for several years and was able to contribute expertise in commercial micropropagation and sales networking for the target crops. The companies cooperated successfully to maximize the benefits of advanced micropropagation methods and to actively market the products of these ventures.

Project Impacts

- ◆ In its first uses of the bioreactor for mass propagation, Fitotek was able to use 32 initial shoots to produce 3,400 harvestable plants with greater consistency of size and color.
- ◆ The bioreactor system was effective for commercial production with the potential of producing pineapple plants 40% more cheaply than previous methods. This system effectively reduced requirements for labor, raw materials, electricity and space.
- ◆ With these encouraging results, Fitotek added several bioreactor units with the capacity of producing 12 million plantlets a year. However, the demand for pineapple declined rapidly during the project, and necessitated a reassessment of this strategy. At the same time, demand for ginger in the country was rising, and Fitotek was able to adapt its micropropagation techniques to produce ginger instead of pineapple.
- ◆ This project illustrates a successful collaboration between private sector partners, and the flexibility of the partners to meet changing market demands.

Maize transformation for development of corn stem borer resistance

Michigan State University, USA

Cornell University, USA

ICI Seeds, USA

Texas A&M University, USA

1991-1996

Project Goal

In the U.S. the European corn borer (ECB) (*Ostrinia nubilalis*) is an important pest of maize, and maize transformation strategies using Bt genes have been highly effective in reducing losses to this insect. In Indonesia a close relative of the ECB, the Asian stem borer (ASB) (*Ostrinia furnacalis*) is the major pest. The life cycle of this insect allows it to threaten maize virtually all year round.

The specific goal of this project was to develop a genotype-independent transformation system for maize, and to produce maize plants resistant to corn stem borer using insect resistance genes. Several partners collaborated in this effort.

- ◆ To develop a biolistic system to engineer maize plants in a genotype non-specific manner, and to use this system to engineer a maize genotype with insect resistance genes. (*Michigan State University*)
- ◆ To develop plasmid constructs containing the Bt genes controlled by a rice promoter. (*Cornell University*)
- ◆ To genetically engineer maize using the *Agrobacterium* transformation system. (*Texas A&M University*)

Project Impacts

Twelve maize lines were initially selected for transgenic research, which were narrowed down to three lines for continued research in the Midwest region of the US.

A novel system for maize transformation using genotype non-specific shoot multiplication was developed (see publications and patents listed below). However, Bt maize lines did not express the toxin protein in sufficiently high levels. The *Agrobacterium* transformation system was not successful.

The need for codon-modified, high-expression genes was noted. Identification of a further private sector partner (Pioneer Hi-Bred) allowed further progress to be made in this area.

Publications

H. Zhong, C. Shrinivasan, and M.B. Sticklen. 1992. Morphogenesis of corn (*Zea mays* L.) *in vitro* I. Formation of multiple shoots clumps and somatic embryos from shoot tips. *Planta* 187:490-497.

H. Zhong, C. Shrinivasan, and M.B. Sticklen. 1992. Morphogenesis of corn (*Zea mays* L.) *in vitro* II. Transdifferentiation of shoots, tassels, and ear primordial from corn shoot tips. *Planta* 187:483-489.

H. Zhong, M. Bolyard, C. Srinivasan, and M.B. Sticklen. 1993. Transgenic plants of *Agrostis palustris* Huds from microprojectile bombardment calli. *Plant Cell Rep.* 13(1):1-6.

B. Sun, S. Zhang, H. Zhong, D. Warkentin, B. Wu, R. Wu and M.B. Sticklen. 1994. Inheritance of a potato proteinase gene in hybrid corn. *J. Plant Physiology*.

H. Zhong, S. Zhang, B. Sun, D. Warkentin, and M.B. Sticklen. 1994. A novel system of maize transformation using the genotype non-specific shoot multiplication. *Bio/Technology*.

S. Assem, H. Zhong, M.B. Sticklen. 1994. Efficient and genotype-independent regeneration of Egyptian maize.

Patents

US Patent No. 5,281, 529 (1994) System to produce ovaries and tassels in vitro, to crossbreed maize under a laminar flow hood, and to grow hybrid corn plants in vitro in an efficient manner.

US. Patent No. 5,320,961 (1994) Transforming maize genotypes via a novel genotype non-specific shoot multiplication system.

Development of Asian corn borer resistance in tropical maize

Garst Seed Company (formerly ICI Seeds, Inc.), USA
Central Research Institute for Food Crops (CRIFC), Indonesia

1995-1998

Project Goals

In the U.S. the European corn borer (ECB) (*Ostrinia nubilalis*) is an important pest of maize, and maize transformation strategies using Bt genes have been highly effective in reducing losses to this insect. In Indonesia a close relative of the ECB, the Asian stem borer (ASB) (*Ostrinia furnacalis*) is the major pest. The life cycle of this insect allows it to threaten maize virtually all year round. The specific goals of this project were:

1. To produce tropical maize with resistance to Asian stem borer (ASB) (*Ostrinia furnacalis*).

Transfer of enabling technologies to Indonesian scientists via training in the US.

Commercialization of insect resistant germplasm generated from the project.

Project Impacts

The Garst/CRIFC collaboration produced was a technical success. Achievements included:

- ◆ Optimization of a cryV gene for expression in maize
- ◆ Demonstrated mortality of the Asian Corn Borer to the cryV protein
- ◆ Development of constructs for maize transformation utilizing the codon-modified cryV gene and the maize polyubiquitin promoter
- ◆ Transformation of tropical germplasm line PN2119 and temperate hybrid line A1888xB73 using the biolistic gun
- ◆ Transformation of the temperate hybrid line A1888xB73 using Garst's "whiskers" technology
- ◆ Field testing of transgenic A188xB73 lines for efficacy against the first and second generation European Corn Borer
- ◆ Of the 248 event-plan combinations, 39 exhibited first generation corn borer resistance as determined by a visual system of leaf damage assessment

Additionally, three Indonesian scientists were trained at Garst for five weeks to learn biosafety and regulatory issues. An additional four Indonesia scientists received technical training lasting from three to 12 months in which they learned:

- ◆ Maize transformation and regeneration
- ◆ Tissue culture of tropical and temperate germplasm
- ◆ Insect bioassays

- ◆ Molecular characterization; i.e. PCR, ELISA, Southern blots, Western blots
- ◆ Field evaluation, artificial infestation and statistical design/interpretation
- ◆ Industrial research and development

However, while a success from a research and capacity building point of view, the project was not ultimately successful in developing tropical maize for use in Indonesia. This was due to primarily policy constraints, but also additional technical constraints. While initially focused on using tropical germplasm, phytosanitary restrictions forced the project to initially transform a temperate line of maize. The legal uncertainty surrounding commercialization of maize developed using the biolistic gun required the use of ICI's proprietary technology, which was only successful in transforming one particular temperate line of maize. This material would have to be backcrossed into tropical maize for development of material suitable for Indonesia. Additionally, the Bt gene, which has been incorporated into the maize, was also proprietary.

At the time of the project, Indonesia did not have in place patent or plant variety protection laws that would protect hybrid seed and transgenic plants. Indonesia still cannot provide adequate legal protection for this material, although they have recently passed a Plant Variety Protection Law. Unfortunately none of these issues were brought to the table when the initial collaboration was undertaken. In this case, partners expressed reluctance to make commitments until the results of the research were known. Thus both the scientific and training component of the project proceeded with great success, but when the scientists returned home, no mechanism existed for them to transfer to their own country the genes and varieties with which they had worked at Garst.

At that time, Indonesia also lacked the appropriate biosafety guidelines or regulations for field-testing of genetically engineered plants, and many companies, as well as the ABSP, are reluctant to test material in countries without adequate biosafety policies. National guidelines were subsequently passed by ministerial decree on September 2, 1997, and with funding from the World Bank and the Indonesian government, construction of a biosafety containment facility began that year. There are currently several field trials of transgenic crops in the country, all of which have been produced by multinational companies.

The patent laws issues in Indonesia are still largely unresolved regarding protection of genes, and if this situation does not change it will continue to inhibit public sector research institutes from accessing proprietary materials from either the public or private sectors outside the country.

Although USAID and ABSP tried to pre-empt the policy issues that would affect the technology transfer process, additional levels of unforeseen detail were encountered that brought the process to a halt. In the case of Indonesia, the biosafety issues have now largely been overcome, but the questions of IPR still have to be resolved. The transgenic material produced during the project is held in trust, however the research contract with Garst has since expired and due to budgetary constraints was not renewed.

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 such 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.

Screening for insect resistance in Kenyan maize

Kenya Agricultural Research Institute (KARI), Kenya
CIMMYT, Kenya

1997-2000

Project Goals

1. To collect local and exotic maize germplasm for use in screening for resistance to Lepidopterous stemborers
2. To elucidate the genetic basis of host plant resistance against *Busseola fusca* and *Chilo partellus* by use of artificial infestation
3. To develop a heterotic population with adequate levels of resistance to stemborers

Lepidopterous stemborers constitute the most widely distributed and serious group of insects attacking maize in Kenya. About 23 stemborer species have been recorded. Amongst them, the most common stemborers are in the spotted stemborer family (SSB), including *Chilo partellus*, *C. orichocalciliellus* and *Eldana sacharina*, Walker. In the *Noctuodae* family are the maize stemborer (MSB) *Busseola fusca* Fuller and *Sesamia calamists* (pink stemborer).

Many strategies to reduce losses due to stemborers, estimated at 23-53%, have been practiced. These include chemical pesticides, cultural and other management practices, including early planting. However, chemical control is not very effective and development of maize varieties with host plant resistance (HPR) is generally considered the most cost-effective method for controlling insect damage in a sustainable agricultural systems. Development of multigenic resistance to stem borer was the main goal of this study.

Project Impacts

Since 1997, 51 local and exotic lines have been collected and planted during the long rainy season. In addition, lines have been obtained from CIMMYT and Cape Town. Of the lines screened for resistance to infestation by *Busseola* and *Chilo*, only two inbred lines showed good tolerance against both *Chilo* and *Busseola* infestation.

In 1998, new sources of resistance were examined in 127 maize lines planted at the Embu main station. Two inbred lines showed acceptable tolerance to both SSB and MSB under artificial infestation.

In 1999, 28 maize accession lines were evaluated. Six lines showed high tolerance to *Busseola* and *Chilo*, while other lines showed moderate tolerance. Days to flowering were not affected.

Data suggest that most of the materials tested could be selected for foliar damage tolerance mainly due to the decrease in tunnel length – a tolerance attribute. Continued work in collaboration with CIMMYT and support from other donors will elucidate the genetic basis of the tolerance.

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.

Transgenic plants carrying the toxin genes of Bt have now been released commercially in many countries around the world and efforts are underway to utilize such plants in Egypt and the Middle

East. The overall objective of the research projects at the University of Texas at Dallas in collaboration with AGERI, Egypt, is to determine the molecular mechanism(s) of insect resistance to the insecticidal toxins (Cry toxins) of *Bacillus thuringiensis*.

At AGERI selection for resistant cotton leaf worm by exposing larvae to Cry1C toxin for 25 generations has given rise to resistant strains showing an increased 10-fold tolerance to Bt toxin compared to the susceptible strain. The protein and DNA profiles of these resistant strains are currently being analyzed. .

Achievements and Impacts

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.

Drs. M. Ibrahim and W. S. A. Maaty received their PhD's under Dr. Lee Bulla's direction at the University of Wyoming and, since, have returned to Egypt where they hold important research positions in the Agricultural Genetic Engineering Institute (AGERI. Dr. Ibrahim is in charge of a research program on microbial insecticides at AGERI and Dr. Maaty is leading investigations in proteomics.

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. 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.

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

1995-2003

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 fifth year of field tests of transgenic potatoes with resistance to Potato Tuber Moth (PTM). The researchers have 2 years worth of data on the resistance of transgenic *Spunta* potatoes, a local fresh market cultivar in Egypt. These *Spunta* lines, transformed with a *cryV* 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 were the most first of any trials in the developing world sponsored by the public sector. 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.

Collaborations with CIP, Peru

The potato project at MSU had and collaborative relationship with the International Potato Center (CIP) to assess the transgenic potatoes developed by MSU researchers. A laboratory study was carried out at CIP to study the larval development of two potato tuber moth species, *Phthorimaea operculella*, and *Symmetrischema tangolias*, which are important pests of potato throughout the Andean region, on the transgenic material from MSU. This *cryV*-Bt gene therefore appears to offer a new and additional source of resistance genes for the Andean potato tuber moth, and can

therefore perhaps be pyramided with other Bt genes for effectiveness toward the development of durable resistance to potato tuber moths and other insect pests. Future work will test mortality of these insects on transgenic tubers, and discussions are currently taking place with CIP to assess the possibility of testing the material in the field.

Collaborations with VOPI, South Africa

In December 1999, an MTA was signed to facilitate transfer of 12 Bt transgenic potato lines (tissue culture plantlets) to South Africa for greenhouse and field evaluations. The Agriculture Research Council of South Africa is currently seeking an import permit from the government to obtain these materials from the ABSP potato project at MSU. Two field trials were planted in South Africa in 2002. One field trial was established at Roodeplaat and the other at Ceres. Both trials were harvested in 2003. Additionally, a storage trial was conducted at Roodeplaat. Six potato lines containing the Bt-Cry1Ia1 gene and two non-GMO controls, Spunta and BP1 were used in all trials. The field trial at Ceres showed complete resistance with no damage to the foliage of any transformed line. The non-transformed controls were severely attacked. The trial at Roodeplaat could not be evaluated due to low tuber moth damage on even the control plants. All but one transformed line exhibited total control against potato tuber moth for at least eight months in open storage. These results from the South African field trials are in agreement with results from the field and storage trials conducted in Egypt in 1999 and 2000.

Collaborations with CRIFC, Indonesia

A Biologically Active Material Transfer Agreement (MTA) between MSU as the supplier of a Bt potato product, and the Central Research Institute for Food Crops (CRIFC) as the recipient, was signed in 1997. Through this MTA, transgenic potato lines of the cultivars Atlantic and Lemhi Russet containing the *cry1Ia1* gene were imported into Indonesia. In 1998, glasshouse tests (with biosafety containment) were conducted on these Bt-potato lines. The results of this glasshouse test showed that the Cry1Ia1 protein was very effective against PTM. The Biosafety Technical Team also evaluated these Bt-potato lines. The next step in the evaluation procedure, contained field testing of the Bt-potato lines, were postponed at that time (1999) due to the intention of Indonesian authorities to revise the Provisions on Biosafety to include food safety assessment. At the same time, new transgenic lines developed by MSU (Atlantic and Spunta) became available. These new lines with higher levels of *cry1Ia1* expression and better resistance to PTM, will be tested as soon as the appropriate regulation requirements are met.

Establishing Licensing Relationships for IP

The ABSP Project at Michigan State University (MSU) licensed the *cry1Ia1* gene from the ICI Seeds Company on October 21, 1994. The gene was licensed for research purposes only to develop transgenic potatoes resistant to the potato tuber moth (PTM). The initial license was for three years and was extended for an additional 3 years (until October 5, 2000) by the Garst Seed Company on October 7, 1997. Syngenta Company provided a further extension until June 2002, and this was then extended until December 31, 2002.

The ABSP project conducted an initial intellectual property (IP) audit of the Bt potato lines and based on this IP audit determined the elements of third party intellectual property used in the material. After a meeting in January 2003 between ABSP, a USAID representative and Syngenta representatives in Basel, Switzerland, Syngenta expressed its intent to grant a royalty free license to the ARC. Syngenta representatives also offered to work with MSU to obtain freedom to operate (FTO) on third party intellectual properties used in the development of the Bt potato product. The MSU Bt potato team will work with the MSU-Office of Intellectual Property (OIP) and other appropriate parties in partner countries to ensure that all the legal and regulatory requirements are met while commercializing this technology.

Project Achievements and Impacts

- ◆ **Transformation:** The development of effective gene constructs for potato transformation with Bt genes for resistance to potato tuber moth—engineered vector constructs and expressed Bt-cry1Ia1 gene in over 200 potato lines.
- ◆ Of particular interest are the potato lines, Spunta G2 and Spunta G3, from a locally grown Egyptian variety transformed with a Cry V vector without the GUS reporter gene that 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, thus easing any concerns about export problems. These Bt-Spunta lines are able to control potato tuber moth damage both in the field and in storage.
- ◆ **Field Testing:** Over 40 different Bt-lines have been field tested in Michigan, Egypt and South Africa over the life of the project. These lines have performed well agronomically, and many have expressed excellent resistance to tuber moth in the field under contained trials.
 - ◆ This project brought about the first field trial of transgenic plants in Egypt in 1997. This field trial was one of the main incentives for establishing the Egyptian national biosafety guidelines for Egypt.
 - ◆ Multiple years of field-testing of transgenic potatoes in Egypt and three years of tests with resistant and susceptible tubers in traditional Egyptian storages have been completed. All studies show nearly 100% control of tuber moth.
 - ◆ First field trial of Bt-cry1Ia1 transformed potato plants was conducted in South Africa in 2001.
- ◆ **Research Linkages:** Effective linkages have been made to international research centers and to other developing country institutions in order to expand the evaluation of material and to analyze potential impact.
- ◆ **Collaboration:** 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. The high level of flexibility within the project has allowed 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.
- ◆ **Commercialization 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.
 - ◆ Food safety assessment of Bt-Spunta lines has been initiated in collaboration with researchers in Germany and AGERI.
- ◆ **Innovative research:** Studies have been conducted to evaluate the effect of combining natural resistance mechanisms with Bt-cry1Ia1. The efficacy of the Bt-cry1Ia1 gene expression against other potato insect pests has been evaluated.
- ◆ **Training:** Egyptian scientists have been trained in techniques of genetically engineering the cultivated potato, conducting field trials under biosafety guidelines, and evaluating insect resistance under field and laboratory environments.
- ◆ The potato group at MSU and its partners in Egypt and South Africa have developed and tested over 200 different potato lines and identify two lines, Spunta G2 and Spunta G3, which have the potential for commercial application. The benefits of this Bt potato to the farmer and end-users will be, reduced input costs (less insecticides purchased), increased marketable

yield, improved quality, reduced post-harvest losses, reduced human exposure to pesticides, and less pesticide residues on potato tubers.

Future Work

This project will continue under the umbrella of ABSP II. In the next phase of the work, the focus will shift from research to efforts to ensure the successful commercialization of at least one of the transgenic potato tuber moth-resistant varieties in South Africa. During the four-year period contemplated for this project, product development and commercialization is likely to be completed in South Africa. It is anticipated that this will provide the template for work in other developing countries such as Indonesia and India, and that some of the data gathered for South Africa (product performance, product quality) will be applicable to variety development in the other countries. Activities included under this component will involve contained on-station field trials, contained on-farm field trials, multi-location field trials, variety development and registration, seed certification, conventional breeding efforts and transformation of local varieties with the *cry11a1* gene. The conventional breeding efforts and transformation of local varieties will provide a long-term supply of commercial varieties, which ought to receive more support from the commercial potato sector. Commercial interests would then be relied upon to bring the new varieties to commercialization, taking advantage of the early USAID investment in food safety assessment and ecological studies. This investment will have maximal benefit for varieties derived through conventional breeding from the first approved Spunta event, but subsequent transformed lines could also rely upon most of the data generated for that first event as well.

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Development of virus resistant cucurbits

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

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 was to develop a novel, non-regeneration based system for cucurbit transformation. To this end two approaches were investigated: one was 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.

Initial results suggested that the electrotransformation procedure was successful when the DNA is directly incorporated into the developing floral. However, further efforts led to the conclusion that electrotransformation and pollen-tube mediated technologies gave sporadically positive, but non-reproducible results.

However, an effective *Agrobacterium*-mediated transformation system was established for cucumber and several genes of interest have been successfully introduced. The *Agrobacterium* system has been used to 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 Raket did not however regenerate well in the *Agrobacterium* system.

An MSU international graduate student from Egypt 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.

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 (see next section).

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. The 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 significant 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. With regard to the question, does pollen-mediated dispersal of transgenes differ from native genes, short and long distance gene movement data validate the assumption that native and transgenes have the same dispersal patterns.

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 R₂ 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.

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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 are:

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 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.

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Whitefly biotypes and biotype-specific transmission of geminiviruses

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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.

Development of insect and virus resistance in sweetpotato

Monsanto Co., USA
Michigan State University, USA
Kenya Agriculture Research Institute (KARI), Kenya
Central Research Institute for Food Crops (CRIFC), Indonesia
International Service for the Acquisition of Agri-Biotech Applications (ISAAA), USA
International Potato Center (CIP), Peru

1992-2000

Project Goal

In Kenya the most important root and tuber crops are potato, cassava and sweetpotato, which is the most widely distributed, and as elsewhere in Africa, sweetpotato is mainly grown by women small-scale farmers. Despite the importance of sweetpotato for smallholder farmers in Kenya, there are serious production problems facing the crop, including pests and diseases and inadequate quantities of good quality planting materials. The major pests include sweetpotato weevils and vertebrate pests. Sweetpotato virus disease (SPVD) is the most important disease of sweet potato in Africa, infected plants yielding less than 50% compared to virus free plants. SPVD is caused by a dual infection with sweetpotato chlorotic stunt virus (SPCSV) and sweet potato feathery mottle virus (SPFMV). SPCSV is transmitted semi-persistently by the whitefly *Bemisia tabaci* and SPFMV is transmitted non-persistently by aphids. Many of the important early maturing and high yielding sweetpotato varieties are highly susceptible to this virus. The overall goal of the project was to develop transformed Kenyan sweetpotato varieties with resistance to Sweetpotato Feathery Mottle Virus (SPFMV) in collaboration with Monsanto, and to transfer the improved varieties to Kenya.

Specific project objectives included the following:

- i. To develop suitable assay systems for virus challenge and protection of coat protein gene transformed sweetpotato,
- ii. To train KARI scientists and technical staff in all aspects of technology development, biosafety evaluation and Intellectual Property Rights (IPR),
- iii. To prepare biosafety application and evaluation structures to enhance the transfer and field evaluation of transgenic sweetpotato in Kenya, and
- iv. To improve production of sweetpotato in Kenya through tissue culture.

Project Impacts

- ◆ One sweetpotato variety was successfully transformed using the SPFMV coat protein gene and has shown good levels of virus resistance in laboratory and glasshouse trials.

- ◆ Regulatory approvals for the field-testing of the sweet potatoes were developed and passed by the national Biosafety Committee. The transgenic sweet potatoes were one of the first products to be reviewed by the Kenyan National Biosafety Committee.
- ◆ Field trials of these transgenic lines were first planted in Kenya in late 2000. These and subsequent field trials have shown promising results for these resistant lines.
- ◆ Research on virus and sweet potato weevil resistance is continuing at KARI and Monsanto.
- ◆ Several Kenyan scientists were trained in tissue culture techniques and in transformation technologies
- ◆ Kenyans researchers and policy makers were trained in the area of IPR and biosafety.
- ◆ Kenyan research capacity was improved, and facilities for laboratory/glasshouse and field biosafety containment were developed and/or upgraded.

This informal arrangement involving ABSP illustrates an example of research and policy collaboration, between Monsanto and the Kenyan Agricultural Research Institute (KARI). Monsanto donated the technology royalty-free for use in sweet potatoes in Africa, effectively removing any intellectual property constraints to transferring the technology to Kenya. ABSP had identified Kenya as a focus country for Africa and identified sweet potato as an important crop for both Kenya and Indonesia. ABSP also supplied Monsanto with information about technology transfer to developing countries. In the process, ABSP supported a postdoctoral researcher at Monsanto and short-term visits of Kenyan and Indonesian scientists to Monsanto. It also funded a biosafety consultant to assist Kenyan scientists in developing a proposal for review by the Kenyan Biosafety Committee and USAID's Biosafety Committee and supported a direct subagreement with KARI to assist in in-country capacity development and technology transfer. At the end of the initial grant, Monsanto continued to support the project from its own resources and from funds provided by several other organizations. ABSP also provided support and training in the setting up of 'mock' field trials in preparation for the actual trials. These biosafety capacity building activities of ABSP and other organizations, including the International Service for the Acquisition of Agri-biotech Applications (ISAAA) have contributed substantially to Kenya's leading position in sub-Saharan Africa in moving forward in the application of biotechnology.

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.

Development of a high beta-carotene variety of mustard for potential deployment in a food based-approach to reduce vitamin a deficiency in India

Monsanto, USA

Tata Energy Research Institute (TERI), India

2000-2003

Project Goal

Vitamin A deficiency is endemic in the developing world, with the result that nearly 800 million people suffer chronic disease and death due to lack of dietary access for this important micronutrient. Most at risk are young children and women of childbearing age. While successful in some developing countries that have adopted a sustained effort, non-food based methods of dietary supplementation have yielded sporadic results. The overall goal of this project was to develop several locally acceptable varieties of mustard oil with enhanced b-carotene levels and with good agronomic characteristics and specifications that would promote adoption by Indian farmers and potentially could function in a food based intervention scheme to alleviate Vitamin A deficiencies in India and to enhance child survival.

Monsanto contributed proprietary technology developed via a biotechnology approach to a technical program, working in close collaboration with an Indian R&D partner, the Tata Energy Research Institute (TERI). Specifics objectives were as follows:

1. Transfer of technology developed for temperate varieties of canola into Indian varieties of mustard;
2. Varietal testing under Indian laboratory and field conditions to assess agronomic performance and nutritional value;
3. Training of Indian scientists in the various techniques and disciplines required to develop this product;
4. Preliminary studies to evaluate the socio-economic use patterns associated with mustard oil as a component of the Indian diet, especially in relation to vulnerable segments of society;
5. Preliminary assessment of intervention mechanisms which might be suitable for the introduction of a beta-carotene enhanced variety of mustard; and
6. An in country workshop to increase awareness about the utility of a food-based, biotechnology approach for nutritional enhancement and to address concerns/issues among various stakeholders.

Research Progress

During the first phase of this project, fifty transgenic seeds and 50 null segregants (T3 generation) of each of five *Brassica juncea* lines (varieties Varuna and Pusa Bold) were transferred by Monsanto to TERI for further research. T3 seeds were grown at the National Phytotron Facility at

the Indian Agricultural Research Institute (IARI), New Delhi, and in the greenhouse at TERIs Gual Pahari campus in Gurgaon. From these seeds, a T4 and then a T5 generation were produced.

Transformation of a yellow-seeded, low erucic acid line (YSRL), using the methodology that was followed for the development of transgenic Pusa Bold and Varuna was also attempted with little success. Because of the difficulty in transforming YSRL, it was decided to discontinue the efforts on this variety and focus attention on generating more transformed lines in Varuna and Pusa Bold.

Socio-economic analysis of mustard oil use and consumption patterns among affected households and communities is necessary to develop an intervention strategy for effective product introduction. Data on the prevalence of vitamin A deficiency, as well as food and oil consumption patterns in various states, was obtained from the reports of National Nutrition Monitoring Bureau (NNMB) and compiled.

Other activities undertaken in Phase I of the project included training of a TERI scientist, in mustard transformation techniques at Monsanto's research facility at Bangalore, the constitution of an advisory committee of prominent Indian scientists to guide the project as well as the convening of an International Symposium on Biotechnology for Food & Nutritional Security, organized by TERI in 2002. This symposium focused on various aspects pertaining to the commercial release of golden mustard. The first phase of the project ended on 30th June 2003.

IPR Agreements and Freedom to Operate

The current research agreement between TERI and Monsanto expires in 2005. TERI and MSU are committed to continue this project to develop, commercialize and deliver a final product. A preliminary inventory of the intellectual properties (IPs) involved in this project identified the following:

- ◆ Mustard Varieties: Pusa Bold, Varuna, and YSRL; The Pusa Bold is a national check variety developed by IARI. Transformation is currently in progress at TERI with the YSRL variety.
- ◆ Genes: Psy, Crt 1, NPT-II
- ◆ Promoter: Napin

In order to commercialize this technology in India, an IPR Freedom to Operate (FTO) and commercialization agreement needs to be negotiated and finalized. Monsanto needs to disclose with TERI and MSU the current status of IP protection and third party agreements related to these intellectual properties. This way TERI, Monsanto and USAID are clear on which technologies belong to Monsanto and which technologies belong to other parties.

Socio-economic Assessment

In order to assess the potential benefits of this technology to producers (farmers) and consumers, a framework was developed for a socio-economic assessment. The framework will include collection of data through primary and secondary sources/means. TERI have focused on the secondary data collection from the existing publications and reports. The adoption and clear benefits of this technology to farmers needs to be thoroughly assessed.

Road-map for product delivery to consumers

TERI have developed several possible strategies so that this technology can reach children and pregnant women who would benefit most from the beta-carotene rich mustard oil. One potential strategy is to deliver the beta-carotene rich mustard oil to school children through the Indian government run "Mid-day Meal Program" in schools. For the benefit of pregnant women a strategy is being developed to distribute the oil through government run "Women and Child Development Clinics" that serve pregnant and nursing women.

Future Work

This project will continue under the management of ABSP II. In Phase II of the project the process will be divided into five components: product development, a socio-economic study including awareness creation through outreach and communication to the public, regulatory file development, product delivery and an IP/freedom-to-operate review:

Product development will involve: field testing the first five lines of transgenic mustard developed in Phase I of the project; developing additional transformants of mustard and evaluating these lines in greenhouse and field conditions; product quality studies to determine agronomic quality and bio-efficacy; determination of the best modes of delivery of β -carotene enhanced oil; and developing a strategy for seed production and marketing.

For this project TERI does not have the capacity to produce the seed and oil for commercial purposes. Therefore, TERI will be the licensor of rights to sell seed of a transgenic mustard line, and companies such as ProAgro and a local oil company can be licensed to sell the seed and produce the oil, respectively.

Public acceptance of a red-colored mustard oil will be encouraged through dissemination of information on health benefits. Acceptance of red color may be enhanced because the red color is preferred for the pickles and curries that will contain this oil. In order to help guide a strategy for public outreach, the field surveys described in the previous section will also collect information on oil color preference. Government and non-governmental organizations will be approached to interact with the public and raise awareness.

The regulatory file will be developed in consultation with the regulatory authorities, and the first priority will be to obtain a clearer picture of the regulatory requirements that may be imposed. The current research agreement between TERI and Monsanto expires in 2005. In order to commercialize this technology in India, a commercialization agreement with Monsanto must be negotiated and finalized.

Indonesia Small Grants Program

The Indonesian Small Grants Program was a component of the ABSP collaboration with Indonesia, supported by USAID/Jakarta. The program, which funded grants up to an equivalent of \$25,000, was open to both public and private sector institutions, and was designed to encourage the development of applied technologies of importance to Indonesian agriculture. Grants were solicited widely across Indonesian research institutes, universities and companies, and reviewed and approved by a Competitive Grants Review Committee. The Review Committee was composed of a co-ordinator from the State Minister for Scientific Research and Development based at CRIFC, and representatives from the industrial crops private sector, the horticultural crops private sector, the estate crops private sector, the university sector, the National Institute of Sciences R&D Center for Biotechnology, and the ABSP core program. The program was managed by CRIFC and the summaries below briefly outline those projects funded by the program. Total funding for the Small Grant Program was \$100,000.

Use of immunoassay probe for detection and monitoring of *Phytophthora spp.*, a causal agent of pod rot of *Theobroma cacao*

Biotechnology Research Unit for Estate Crops, Indonesia

Project Goal

Cocoa is an important crop in Indonesia with more than 58% of the 221,000-ton total production coming from smallholdings. The existing plant materials in Indonesia and other cocoa producing countries are susceptible to pod rot disease caused by *Phytophthora* spp. Forty percent of pod loss is usually from damage due to the disease and chemical control is currently the major control strategy. Fungal propagules in the soil are the primary cause of infection, but there are no techniques available to quantify the level of inoculum in the soil. Immunoassay has the potential to detect and quantify the pathogen population in the soil.

The objectives of this project were as follows:

1. To investigate if *Phytophthora* associated with cacao in Indonesia consists of several serotypes.
2. To produce polyclonal antibodies (PcAbs) against *Phytophthora* spp. associated with cocoa pod rot disease.
3. To investigate the potential of these antibodies for assessing inoculum potential of *Phytophthora* spp. in soil

Project Impacts

- ◆ The polyclonal antibody developed in this study was highly specific to the *Phytophthora* species associated with pod rot disease of cacao and did not cross react to other soil microorganisms.
- ◆ The antibody was further characterized and found to be bound to polysaccharides with molecular weights of 36, 25, 20 and 17 KDa.
- ◆ Dot Blot Immunosorbent Assay (DIBA) was determined to be an improved method for the detection and monitoring of *P. palmivora*, causal agent of pod rot disease of cacao.

Regeneration study of Indonesian sweet potato

Research Institute for Food Crops Biotechnology (CRIFC), Indonesia

Project Goal

Problems in sweet potato production in Indonesia are caused mainly by sweet potato weevil, and conventional breeding methods have so far failed to produce resistant material. The application of recombinant DNA technology therefore has potential to address this problem, however, this has been hampered due to the inability to regenerate plants efficiently.

The objective of this project was therefore:

1. To study the *in vitro* culture ability and regeneration of seven Indonesian sweet potato cultivars.

Ginger micropropagation using bioreactor system

PT Fitotek Unggul, Indonesia

Project Goal

Ginger (*Zingiber officinale* Rose) is a herbaceous plant indigenous to Indonesia and widely spread throughout India and China. Ginger rhizomes are used as spices, in herbal medicine, and as raw material in the food, beverage and pharmaceutical industries. The demand for fresh and dry ginger and its essential oil on the world market is high both in domestic and international trade. Ginger is propagated through rhizomes and farmers usually take planting material from their own product, a practice that tends to spread diseases. Micropropagation methods have been developed for ginger and have made possible the supply of disease-free material year round. However, solid culture systems are limited in their ability to produce material in bulk, and therefore a bioreactor system of agitated liquid culture is recommended.

The main objective of this project was:

1. To investigate the feasibility of producing ginger using a micropropagation bioreactor system.

The development of CVPD free citrus seedling from protoplast fusion and embryogenic callus cultures

Gadjah Mada University, Indonesia

Project Goal

Citrus greening disease is a major cause of crop and tree loss in many parts of Asia and Africa. Before it was identified as one disease, it was known by various names: yellow shoot (huanglungbin) in China; likubin (decline) in Taiwan; dieback in India; leaf mottle in the Philippines; citrus vein phloem degeneration ((CVPD) in Indonesia; and yellow branch, blotchy-mottle, or greening in South Africa. As it became clear that all these were similar diseases the name "greening" has been widely adopted. Losses due to greening are not easy to assess but are high in many citrus growing areas. Sometimes only sectors of a tree are affected and losses are small, but in other cases the entire tree is infected and crop loss is total. No detailed loss

studies have been published, but in Indonesia not less than 3 million trees were destroyed between 1960 and 1970, with groves in most regions of Java and Sumatra being abandoned by 1983.

Greening is caused by an unculturable Gram-negative phloem limited bacteria belonging to the alpha subdivision of the *Proteobacteriaceae*. The 16S rDNA comparative studies led to the proposed classification of the causal agent as a "*Candidatus*" with generic name *Liberobacter*, as defined for uncultured organisms. Two distinct species have been identified based on sequence comparison and the names, *Liberobacter africanum* and *Liberobacter asiaticum*, have been proposed for the African and Asian greening organism respectively.

The objective of this project was to:

1. Use tissue culture techniques to obtain citrus seedlings free of the CVPD agent.

Project Impacts

- ◆ The plant material that gave the best explant material for embryogenic nucellus cell culture was determined to be immature fruits.
- ◆ Optimal culture media for callus culture and regeneration were identified for several citrus varieties.
- ◆ Further funding for this project was obtained from The Indonesian Directorate General of High Education.

Biosafety

Although biosafety was not a high priority among the project's developing country collaborators at the inception of the ABSP, it is now one of the major issues 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 places a high priority on the establishment of science-based regulatory structures for the promotion of technology access for developing countries. 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. More recently the ABSP has begun exploring the feasibility of regional regulatory frameworks in eastern and southern Africa. The success of this assistance as described below, linked to the ABSP research program, is one of the major achievements of the ABSP.

Selected Activities

- ◆ **Latin America & Caribbean Region Biosafety Workshop, Jamaica, May 1993.**
ABSP organized a regional workshop in Jamaica, in collaboration with the Bean/Cowpea CRSP in May 1993. 42 representatives from 12 countries attended the workshop the objectives of which were: i) to examine the status of biosafety guidelines and regulations in the region for testing and utilization of genetically engineered food crops; and ii) to assist participants in developing work plans and recommendations form which to begin building the necessary biosafety policies and guidelines in their own countries. Proceedings of the workshop were published. [*Proceedings of the USAID Latin America Caribbean Region Biosafety Workshop, May 10 - 13, 1993, Oracabessa, Jamaica.*]
- ◆ **Biosafety Internship Program: Guidelines Development, MSU, May-July 1993.**
ABSP organized an eight week internship 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, Kenya and Indonesia participated in the ABSP Biosafety Intern Program, May-June 1993. The Indonesian scientists then participated in a *hands-on* biosafety training program at ICI Seeds, Iowa, while the Egyptian and Kenyan Scientists participated in a program at Michigan State University. The scientists then reconvened in Washington DC where they had the opportunity to interact with federal personnel at USDA/APHIS, FDA and EPA responsible for various aspects of biosafety.
- ◆ **Genetic Resources Workshop, Egypt, June 1994.**
The ABSP/AGERI project, in cooperation with Genetic Resources Communication Systems (GRCS), Inc., the Egyptian National Research Program, and USAID/Cairo held a two day Genetic Resources Workshop in Cairo. The workshop brought together experts from Egypt and from the international Community to discuss various issues

related to genetic resources in Egypt and the region. A special Issue of *Diversity* journal focusing on this workshop was published. The Mediterranean issue of *Diversity* was translated into Arabic and 3,000 copies of this version were distributed.

- ◆ **Biosafety Workshop at AGERI, Egypt, January 1994.**
The goal of this workshop was to create a greater awareness and strengthen the biosafety regulatory framework in Egypt and the Middle East. The workshop involved international experts on in biosafety, and scientists and regulatory personnel from Egypt and selected countries in Africa. The workshop addressed policy, risk assessment and field-testing issues surrounding the management and safe handling of transgenic plants. The proceedings were published [*Biosafety/Intellectual Property Rights Project Evaluation, Proceedings from the AGERI & ABSP Workshop Series January 24 - 31, 1994, Cairo, Egypt.*]
- ◆ **Consultations on Egyptian Biosafety Guidelines, 1994.**
Dr Patricia Traynor reviewed the Egyptian draft biosafety guidelines and provided comments.
- ◆ **Consultations on Indonesian Biosafety Guidelines, 1995.**
Dr Patricia Traynor worked as a special consultant in assisting the Indonesian biosafety guidelines drafting committee.
- ◆ **Biosafety Internship Program: Risk Assessment and Risk Management, MSU, August 1996.**
ABSP and the Institute of International Agriculture at MSU in cooperation with the Information Systems for Biotechnology Program at the Virginia Polytechnic Institute (VPI), organized a 2 week internship program in biosafety risk assessment and management. 15 international participants attended this program, the goal of which was to assist developing countries in the field-testing of transgenic crops. The internship covered the theory and practice of risk assessment and management in agricultural biotechnology programs.
- ◆ **Construction of Containment Greenhouse Facility at AGERI, Egypt, 1995.**
ABSP provided leadership in the development of a cooperative sub agreement 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 who stated in his report that “the biocontainment greenhouse facility at AGERI meets the international standards for growing genetically engineered organisms, and is ready for commission.”
- ◆ **Assistance to Indonesia in Developing Biocontainment Facilities, 1997.**
The ABSP project, through an appropriate intellectual property rights agreement, facilitated the sharing of the plans for the AGERI biosafety greenhouse facility with collaborators at CRIFC in Indonesia. A similar facility was then built in Indonesia through a World Bank loan.
- ◆ **Assistance to Morocco in Developing Biosafety Policies, 1997.**
A colloquium on biosafety, held in conjunction with a workshop on the implementation of Plant Variety Protection in Morocco, was organized on March 24-26, 1997 in Rabat, Morocco. Over 250 participants from various public and private sector institutions in Morocco attended, including participants from the Ministry of Agriculture, Minister of Public Health, Ministry of Commerce, and the Ministry of the Environment. While mainly

focusing on Plant Variety Protection and other IP issues, the colloquium did focus on biosafety issues on the last day of the conference. International experts from USDA/APHIS, the EPA, and the private sector provided their perspectives on the development and deployment of biosafety procedures. Following the IPR/Biosafety workshop, three Moroccan received additional training in the US in August 1997. This training was provided by USDA/APHIS in Washington, DC and included examples of risk assessments, required regulatory testing and certification, as well as export/import issues surrounding the use of transgenic seeds. Following this training, Dr. Pat Traynor, the ABSP's Biosafety consultant, traveled to Morocco in May 1999 to present a paper entitled Biosafety in Agriculture: Ensuring the Environmentally Responsible Use of Biotechnology Products at a conference at Al Akhawayn University, Ifrane, Morocco. She also visited with Moroccan officials to discuss responsibilities and progress in developing biosafety guidelines. To date, Morocco has not yet developed Biosafety guidelines or procedures. ABSP also provided more in-depth training to several Moroccans in the US, where they visited the USDA, EPA and FDA to learn about the US regulatory system.

◆ **Public Awareness/Acceptance of Biotechnology in Indonesia**

Over the past five to six years, ABSP has assisted a number of CRIFC faculty and staff in both biotechnology technical training and in regulatory and intellectual property policy training. This training has provided the researchers at CRIFC with sound scientific knowledge to engage in current biotechnology education and awareness campaigns throughout Indonesia. Teams of scientists recently traveled in Central Java, West Java and Bali to present seminars on the genetic engineering for crop improvement and Indonesia's regulatory system for GMOs. The audience was primarily university and research institute personnel. CRIFC plans to expand this effort to include East Java and will conduct training to educate additional scientists on communicating biotechnology issues and policy to the Indonesian public.

◆ **Assistance to Indonesia in Developing National Biosafety Guidelines**

Indonesia has been a major focus country for ABSP's capacity building in biosafety and intellectual property rights. In 1995, ABSP began providing Indonesia with assistance in developing its national biosafety guidelines.

- ◆ A consultant from the USDA National Biological Impact Assessment Program worked as a special consultant for ABSP and assisted the committee formed by CRIFC in drafting the guidelines. Indonesian experts in each of three research sectors (plants, animals, and microorganisms) were selected as the writing committee with the approval of Indonesia's Ministry of Agriculture. A first draft was produced and entitled "*Guidelines for Planned Introductions into the Environment of Organisms Genetically Modified by Recombinant DNA Techniques.*"
- ◆ In order to improve upon this first draft, CRIFC and ABSP organized a biosafety workshop, held in May 1996, and a total of 45 participants from both the public and private sector attended. Based on the workshop, a new draft was produced, and the guidelines for biosafety were proposed as the basis of a decree from the Minister of Agriculture.
- ◆ A second workshop was then held to finalize the second draft, which was reviewed by Indonesian officials and the Bureau of Law at the Indonesian Ministry of Agriculture.
- ◆ National guidelines were subsequently passed by ministerial decree on September 2, 1997
- ◆ Field tests were approved in 1998 by the National Biosafety Committee for Bt cotton, Bt corn, Roundup Ready cotton, Roundup Ready corn, and Roundup Ready soybean. Multi-locational, unconfined trials have been conducted.

◆ **Development of Biotechnology Initiative with ASARECA, 1999-2003.**

In 1999 ABSP entered into a formal, contractual collaboration with the Association for Strengthening Agricultural Research in Eastern and Central Africa (ASARECA). As part of the process to develop a regional initiative in biotechnology and biosafety, ABSP assisted ASARECA in established a working group (WG) to examine issues and pragmatic approaches for integration of biotechnology through the existing regional networks and for the expansion of regional biosafety regulatory policy development. ABSP provided further technical support to the Biotechnology WG throughout the planning process. ABSP contracted with ISNAR to assist in this process.

- ◆ **ABSP Technical Support.** In order to assist ASARECA, ABSP provided technical support to the WG throughout the planning process. This will ensure that the WG members have access to international expertise in agricultural biotechnology and biosafety.
- ◆ **Biotechnology Inventory.** In 2000 ABSP developed *An Inventory of Agricultural Biotechnology for the Eastern and Central Africa Region*. The report highlighted the current status of biotechnology applied to crops within ASARECA networks in order to give the working group an indication of the future potential of biotechnology tools for the improvement of crops that are important to Africa. The report continues to be highly referenced document. In addition to being available for download from the ABSP website at (<http://www.iaa.msu.edu/absp/inventory1.html>), it was also published in 2001 as part of the USAID Africa Bureau's SD Publication Series. Over 1,100 copies of the report have been downloaded from the ABSP website, and it has been widely praised by public and private sector organizations within Africa
- ◆ **Commissioned Papers on Biotechnology and Biosafety.** In 2001 ABSP assisted ASARECA both in the development of the terms of reference for commissioned papers, one on biotechnology in the region, and one on biosafety. These commissioned papers provided a valuable basis for discussion of the important issues in the subsequent Biotechnology working group meetings and planning activities.
- ◆ **ASARECA biotechnology and biosafety proposal drafted.** A final proposal to be submitted to major donor organizations is currently (Sept 2003) being developed and will include clear links to, and areas of support from the ISNAR-led Program for Biosafety Systems (PBS) and the Agricultural Biotechnology Support Project (ABSP-II) managed by Cornell University.

◆ **Development of Southern Africa Regional Biosafety (SARB) Program, 2000-2001.**

The overall goal of the Southern Africa Regional Biosafety (SARB) program was to conduct regional training related to biotechnology regulation as a means of establishing a foundation for more sound regulatory development and implementation. By taking a regional approach, the program aimed to provide a foundation for later discussions of regulatory harmonization within SADC. SARB acted to promote science-based regulatory implementation and market access for biotechnology applications from both the public and private sectors. The program focused on seven SADC countries: Zambia, Zimbabwe, Mozambique, Mauritius, Namibia, South Africa and Malawi. The Vegetable and Ornamental Plant Institute (VOPI) of the Agricultural Research Council in South Africa is the lead contractor on the program. Innovation Biotechnology, a private consulting firm headed by Ms. Muffy Koch, was also involved in the program as a sub-contract to VOPI.

The major activities and achievements of the program are outlined below:

- ◆ **Activity 1--The establishment of a Regional Working Group of delegates from Core Target countries:** One of the major accomplishments of SARB was the establishment of a regional Working Group (WG) following the first meeting in

Pretoria, South Africa in November 2000. In order to maintain a connection between members of the WG and improve communication a regular newsletter was distributed which proved to be very successful. A second meeting of the Working Group was held in December 2002. The WG formed the basis of a successful network within the SADC region and any future activities should use this foundation. As part of the final WG meeting a symposium “*Strengthening Bioasfety Capacity for Development*” was held in June 2003 in South Africa. This symposium was organised to review the impact of SARB and other ABSP programmes and was attended by 38 delegates from both target and non-target countries as well as representatives from other international programmes.

- ◆ **Activity 2--Regional Workshop on Biosafety:** This workshop, held at the ARC Central Office in Pretoria in March 2001, and attended by 37 delegates from 11 countries, served as a general awareness-raising event on biosafety in the SADC region. It was targeted towards legislators/policy makers, regulators, members of biosafety committees as well as delegates to the Cartagena Protocol on Biosafety and Codex.
- ◆ **Activity 3--Regional Biosafety Training Course:** The purpose of this training course was to train regulators and reviewers in biotechnology and biosafety issues. Held in November 2001 at the ARC Central Office in South Africa, it was attended by 24 delegates from the seven core countries. The course allowed for the first practical evaluation of the new ABSP *Workbook for Technical Training: Biosafety and Risk Assessment in Agricultural Biotechnology*, which has subsequently been used as the basis of in-country training of scientists. Two further regional courses were held: in February 2003 in Pretoria (Botswana, Lesotho, Swaziland and Seychelles), and the second in May 2003 for scientists from Malawi, Namibia and Zimbabwe.
- ◆ **Activity 4--Journalists/Media Course:** The purpose of this workshop was to provide balanced information on biotechnology and biosafety to key individuals in the media in target countries, and to address issues of how policy makers/regulators convey issues of safety and regulation to the media. The workshop was held in South Africa in May 2000 and was attended by 17 journalists from seven countries attended the workshop.
- ◆ **Activity 5--National Follow-up/In-country Biosafety Training:** National-level biosafety training was held as a subset of the target countries to broaden the range of policy makers with biosafety training to include all members of National Biosafety Committees and other stakeholders such as Ministries of trade, industry, farmers organizations, etc. Under this activity SARB granted Namibia financial support to test and verify their proposed biosafety regulations through the drafting of three “applications” for submission to and evaluation by their National Biosafety Forum.
- ◆ **Activity 6--Risk Assessment Research-Sorghum Gene Flow Case Study:** A critical component of biosafety risk assessment and management is knowledge about environmental risks specific in the region, such as gene flow from biotech crops to related African species. Although no suitable GMO sorghum could be located two trials were designed incorporating conventional seed to measure geneflow: one trail to monitor pollen dispersion was planted at the ARC-Roodeplaat in December 2002. It was found that pollination rate dropped off significantly with distance from the pollen source with very little pollination occurring beyond 200m. The second trial was conducted in Bulawayo, Zimbabwe, in collaboration with ICRISAT. Using two cultivars with different seed colour it was shown that hybridization does occur when planted within a short distance of each other.
- ◆ **Activity 7--Core Group Biotechnology Field Trip:** The SARB program sponsored a site visit to China to enable delegates to examine other regulatory systems in place or under development. The tour took place from 7 to 15 August 2002 with fifteen

delegates from the seven countries, accompanied by Dr. G. Thompson and Mr. G. Bothma of the ARC-Roodeplaat. Participants gained insight into the regulatory process and biotechnology research in China.

◆ **Food safety consultancy to Egypt, 2001.**

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

◆ **Development of Biosafety Training Workbook, 2003.**

ABSP developed a manual for biosafety training: **Biosafety & Risk Assessment in Agricultural Biotechnology: A Workbook for Technical Training**. Patricia L. Traynor, Robert J. Frederick, Muffy Koch, Published by The Agricultural Biotechnology Support Project (ABSP), Michigan State University. (ISBN: 1-56525-016-8). Designed to complement technical biosafety-assessment training courses in developing countries, this workbook provides a background for the practical application of biosafety review procedures using a case study approach. The intended audience includes members of national biosafety committees, biotechnology regulatory officials, and scientists working in the public and private sectors. It is a useful resource for national decision-making bodies, government regulators in related areas, and those charged with monitoring approved field-test releases. The workbook is the product of biosafety experts with years of experience in technical as well as information-oriented training. It is organized in three parts.

The workbook was successfully used at the SARB regional training course in November 2001 and during subsequent in-country training courses presented in SARB target countries. Although initially designed to accompany training workshops conducted under SARB; it has been quickly taken up and used in training events sponsored by other capacity building programs, including those of USDA and ISNAR, and will be used in the newly implemented USAID Program for Biosafety Systems. Since publication in January 2003, approximately 250 print copies have been distributed to individuals world-wide; and another 350 used in training courses. In addition, the full text is freely available for download from http://www.ija.msu.edu/absp/biosafety_workbook.html, and this web page has been accessed over 2,000 times since January 2003. Currently the workbook is being translated into French, Portuguese, and Spanish for use in those world regions. Translation into additional languages, including Indonesian is under consideration. Although there are no plans to print these versions, they will also be available to download from the ABSP Website. The workbook's authors are also in the process of developing a companion "teachers" volume, which will be available in electronic form.

Impacts

Indonesia

Indonesia has been a major focus country for ABSP's capacity building in biosafety. Through workshops, internships, and consultants, Indonesian scientists and policy makers have been brought together to address regulatory issues relating to the testing and commercialization of transgenic crops. Along with Egypt, Indonesia has made significant progress in the development of biosafety guidelines and procedures.

◆ **Biosafety Guidelines Developed and Approved**

National biosafety guidelines were passed by ministerial decree on September 2, 1997, allowing Indonesian scientists and international companies and research institutions to field test transgenic crops in Indonesia.

◆ **Transgenic Crops Commercialized.**

Indonesia has the appropriate regulatory policy framework in place for the development, field-testing and commercialization of transgenic crops. The government of Indonesia has already approved the commercialization of insect resistant Bt cotton in selected districts of the country. The Proponent of Bt cotton submitted a written application to the Regulatory Authority in 1998 and after the Technical Team evaluated the documents and the questionnaires, biosafety containment and isolated field tests of Bt cotton were conducted during 1998/99. The BFSC recommended that the Bt cotton product was safe to be planted in the environment and subsequently, multi-location tests of Bt cotton were conducted in 7 locations in the South Sulawesi Province. The Evaluation and Release Variety Team evaluated the results of multi-locational trials of Bt cotton, and a limited permit cotton was granted by the Minister of Agriculture in 2001. A limited permit allows the Bt cotton to be planted only in seven districts of South Sulawesi province. The performance and the possible effects of Bt cotton are evaluated annually. In 2002, about 2700 farmers commercially planted Bt cotton on approximately 4,000-5,000 ha in South Sulawesi in Indonesia.

A Biologically Active Material Transfer Agreement (MTA) between MSU as the supplier of a Bt potato product, and the Central Research Institute for Food Crops (CRIFC) as the recipient, was signed in 1997. Through this MTA, transgenic potato lines of the cultivars Atlantic and Lemhi Russet containing the cry1a1 gene were imported into Indonesia. In 1998, glasshouse tests (with biosafety containment) were conducted on these Bt-potato lines. The results of this glasshouse test showed that the Cry1a1 protein was very effective against PTM. The Biosafety Technical Team also evaluated these Bt-potato lines. The next step in the evaluation procedure, contained field testing of the Bt-potato lines, were postponed at that time (1999) due to the intention of Indonesian authorities to revise the Provisions on Biosafety to include food safety assessment. At the same time, new transgenic lines developed by MSU (Atlantic and Spunta) became available. These new lines with higher levels of cry1a1 expression and better resistance to PTM, will be tested as soon as the appropriate regulation requirements are met.

◆ **GMO Food Safety Guidelines Developed.**

Supported by ABSP, Dr. Muhammed Herman and Dr. Achmad Hidayat, Central Research Institute for Food Crops (CRIFC) attended the International Food Safety course at Michigan State University (MSU) in 1999. Drs. Herman and Hidayat were subsequently appointed to the committee charged with drafting Food Safety Guidelines for GMOs in Indonesia. The Ministry of Agriculture and other relevant ministries in Indonesia have since approved these guidelines.

Egypt

◆ **Biosafety Guidelines Developed and Approved**

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. Impacts of the ABSP have included:

- ♦ Training of the AGERI Biosafety Officer assigned with drafting biosafety guidelines for laboratory, greenhouse and field experiments
- ♦ Construction of a biocontainment greenhouse facility
- ♦ 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 were established in 1998 by Ministerial Decree No. 1648. Development of food safety laws/regulations is currently underway.
- ♦ Field-testing of GMOs, including insect-resistant potatoes (MSU and AGERI), virus resistant squash (AGERI) and virus resistant tomatoes (AGERI) and Bt resistant maize (Fine Seeds/Novartis).

Kenya

Kenya received less financial support for policy and research efforts compared to Egypt and Indonesia, primarily because USAID/Nairobi did not commit additional funds to the program. However, USAID/Africa Bureau did provide a small amount of additional funding for research and policy efforts that assisted the ABSP in providing training and support to Kenya for biosafety regulatory development. Even so, Kenya has made significant progress in the development of its regulatory system. Kenya has:

♦ Instituted a National Biosafety Committee in 1996.

The Kenyan NBC began implementation of biosafety review processes in 1997.

♦ Field-tested transgenic sweet potatoes in early 2001.

This was the first field test of a transgenic crop developed in collaboration with the public sector in Sub-Saharan Africa (excluding South Africa). The trials have since been repeated and further research continues on these resistant lines.

♦ Other Transgenic field trials in Kenya

The National Biosafety Committee has since approved contained field trials of other transgenic crops in the country, including Bt maize and Bt cotton.

South Africa

♦ SARB--Impacts on public awareness/acceptance.

Following the successful workshop for journalists from the region held in May 2001 in Pretoria, two one-day workshops have been held in Malawi and Zambia for local journalists.

Local journalists have received accurate information on biotechnology and biosafety.

- ♦ Positive articles and interviews were included in the local press, radio and television. Comments on these were that they were well informed, balanced and captivating.
- ♦ Public awareness and understanding of biotechnology within the region has increased due to the workshops and subsequent media coverage.

◆ SARB--Impacts on policy.

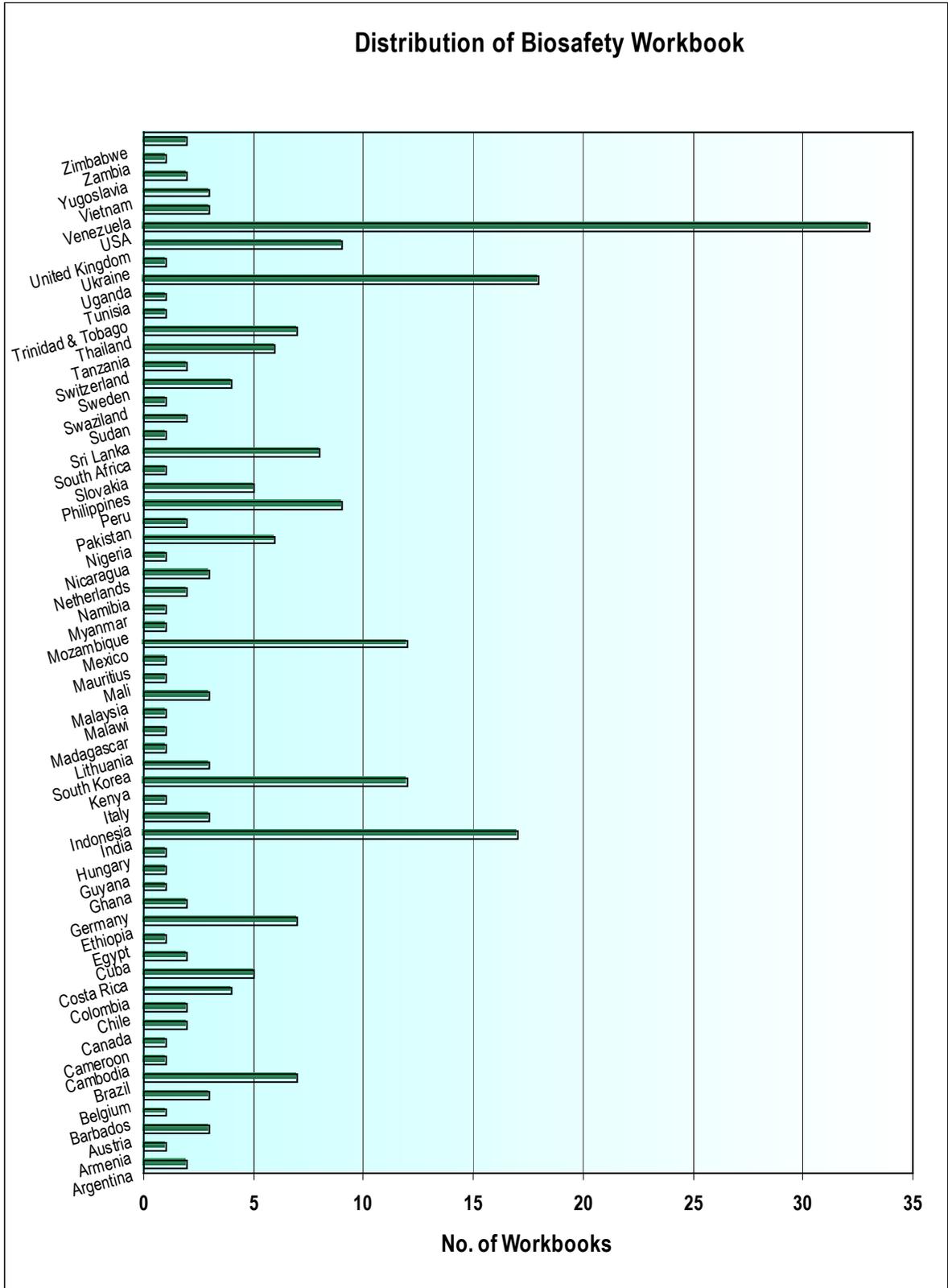
The early activities of the SARB project have initiated discussion on biosafety policy in Mozambique and Botswana and have given impetus to regulation development in Malawi, Mauritius, Zambia and Namibia.

- ◆ A framework for inter-departmental biosafety discussions in South Africa has been established.
- ◆ Priority setting on biosafety implementation in Zimbabwe has begun.
- ◆ Consensus on development of a regional biosafety initiative that could minimize duplication, allow capacity sharing and build confidence in decision making in the region has come out of the March 2002 regional meeting.
- ◆ One-day workshops for policy and decision makers were held in Malawi and Zambia.
- ◆ Important policy and decision makers in Malawi and Zambia are better informed about biotechnology and biosafety.
- ◆ A two-day workshop was held in Mozambique for 56 representatives from 10 provinces for decision-makers, government officials, scientists and members of civil society.
- ◆ Legislation currently being developed for the safe implementation of GM products will be informed through the general introduction to biotechnology and biosafety presented at this two-day workshop.
- ◆ Decision-makers and government officials have been aided in establishing the roles each has to play in ensuring legislation is developed for the safe implementation of GM products.

◆ SARB--Impacts on risk assessment.

The number of scientists within the region with knowledge of how to assess the risks of GMOs has greatly been increased through the training provided by SARB. A six-day regional training workshop was held in South Africa in November 2001 when the ABSP biosafety training workbook was used for the first time with great success. This was followed up with a 2-day workshop in Malawi and a 3-day event in Zambia. Experience has now shown that a 3-day workshop is the best option.

- ◆ More than 50 scientists now have experience in risk assessment.
- ◆ 56 participants in Maputo, Mozambique have been informed about the role of risk assessment in regulatory legislation.
- ◆ The ABSP biosafety training workbook has been successfully piloted with developing country audiences and revisions have been implemented to reflect feedback from the participants.



Technology Transfer/IPR

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.

Several laws and court decisions have contributed to an evolving partnership between the public and private sectors. This partnership has particular relevance for developing country activities in biotechnology. For example, the 1986 Federal Technology Transfer Law has directly affected agricultural research initiatives at land-grant universities and other publicly supported institutions. This legislation responded to the perception that federally supported research results were not being readily adopted by the private sector in the development of new products for consumers and farmers. This act authorized government-supported agencies and institutions to enter into co-operative research agreements with private US companies. The law was developed to bridge the gap between the fundamental research undertaken by public institutions and the more downstream, applied research performed by the private sector. This has led to universities filing for patents as a means to transfer technology to companies efficiently.

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 privatization 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. The ABSP philosophy is 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 our developing country collaborators. Additionally, we've sponsored participation to international meetings and access to news and information on IPR via the World Wide Web. The results and impacts described below speak for themselves. 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.

Selected Activities

- ◆ **Intellectual Property/Patent Internship Program, Stanford University, April 1993.**
An IPR internship program was designed and implemented by Professor John Barton of Stanford Law School from April 1-30, 1993. Seven interns from Egypt, Kenya and Indonesia participated in the program, the goal of which was to provide hands-on experience to legal and scientific personnel from developing countries in various issues related to intellectual property rights. In addition the internship enhanced communication between those involved in the sciences and those with responsibilities in the legal issues surrounding biotechnology. The program encouraged the assessment of current intellectual property structures within the participants' home countries, provided access to literature and expertise regarding IPR in both the public and private sectors.

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- ◆ **Workshop On Intellectual Property Rights, Patents & Licensing, Egypt January 1994.**
This workshop, designed by Prof. John Barton, George E. Osborne Professor of Law at Stanford Law School, was held in Cairo, Egypt from January 24-25, 1994. Over 100 participants from various public and private sector institutions attended the workshop, 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. Proceedings of this workshop were published.

 - ◆ **IPR Workshop, Washington DC, July 1994.**
ABSP sponsored this workshop in Washington DC from July 11-14, 1994 as a follow up to the Egypt workshop. Forty-four participants attended from Egypt, Kenya, Indonesia and Costa Rica, Thailand, Sri Lanka as well as a number of institutions and agencies such as USAID and the World Bank. The purpose of the workshop was to present intellectual property rights in biotechnology as an important issue to institutions and individuals. Proceedings of this workshop were published: *Intellectual Property Rights, Proceedings from the ABSP Workshop Series July 11 - 14, 1994, Washington, D.C.*

 - ◆ **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.

 - ◆ **Plant Variety Protection and Patents Workshop, Indonesia, 1996.**
ABSP, through the support of USAID/Jakarta organized a two-day workshop on intellectual property rights in agriculture from March 25-26, 1996, which was attended by fifty senior representatives from the government and private sector in Indonesia. The workshop was organized in collaboration with the Central Institute for Food Crops (CRIFC) in the Indonesian Ministry of Agriculture. The main goal of the workshop was to assist Indonesia in drafting their new plant variety protection law.

 - ◆ **IPR and Technology Transfer Internship Program at MSU, 1996.**
A two-week internship program in IPR and technology transfer was organized at MSU from February 4-17, 1996. This was organized by ABSP in cooperation with the Office of Intellectual Property and the Institute of International Agriculture at MSU. The goal of the program was to provide hands-on experience to international scientists, administrators and policy makers in the day to day handling of intellectual properties within the context of recent changes in the GATT agreement. To foster networking, the participants also attended the annual meeting of the Association of University Technology Managers (AUTM) in South Carolina.

The success of ABSP's first internship program led MSU to develop it into a short course that has now been offered annually since 1996. The course is run in collaboration with the Institute of International Agriculture and the Office of Intellectual Property. The focus of this one-week program is on IPR and technology transfer education with special emphasis on day-to-day handling and management of intellectual properties as it relates to agriculture. We believe that this is still the only structured short course held in the US that covers the IPR and technology transfer issues related to agriculture.
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During the last five years, 84 international participants have attended this program. The ABSP project has directly sponsored participants from the following countries: Costa Rica (1), Egypt (4), Kenya (2), Morocco (5), Indonesia (6), South Africa (1), India (2) and Ethiopia (1).

- ◆ **Plant Variety Protection and Patents Workshop, Morocco, 1997.**
ABSP through support from USAID/Rabat and the Ministry of Agriculture in Morocco organized a colloquium in Rabat from March 24-25, 1997. The goal of the colloquium was to assist Morocco in the Implementation and enforcement of a new PVP law that was pending approval from the Moroccan parliament. Over 250 representatives from governmental agencies and the agribusiness community attended this colloquium along with 12 international experts from the US and Europe. The proceedings of this workshop were subsequently published by ABSP.
- ◆ **Assistance to Moroccan PVP Office, 1997.**
After the above workshop, three Moroccans from the DPVCTRF (the organization charged with implementing the Plant Variety Protection law) participated in the MSU IPR Management course in July 1997. Additionally, the Moroccans received additional training from the Plant Variety Protection Office of USDA in Washington DC in August 1997. Following this training, the ABSP provided logistical support to purchase computer equipment and software to outfit the new PVP office within the DPVCTRF.
- ◆ **East Africa IPR Workshop, Uganda, 1999.**
ABSP held a workshop on *The Impact of Intellectual Property Rights on International Trade and Agriculture in East Africa* in Kampala, Uganda from January 18-20, 1999. The Ugandan Council for Science and Technology (UNCST) assisted ABSP in the local organization of the workshop. Additional funds for the support of regional participants to attend the meeting were obtained from the Technical Center for Agricultural and Rural Cooperation (CTA, Netherlands), the Rockefeller foundation and Monsanto. Over 70 participants attended the workshop from Ethiopia, Kenya, Nigeria, Tanzania, Uganda, Zambia, Zimbabwe, Switzerland, United Kingdom, France, United States, Costa Rica, South Africa, and the Netherlands.
- ◆ **Intellectual Property Rights Policy Assistance to the Bean/Cowpea CRSP, 2000.**
With ABSP assistance and consultation, the USAID-supported Bean/Cowpea Collaborative Research Support Project (CRSP) developed an IP policy – the first time a CRSP project has institutionalized an IP policy with their U.S. and overseas collaborators. The Bean/Cowpea CRSP Technical Committee reviewed the draft policy and it is currently under consideration by the Bean/Cowpea CRSP community. The adoption of a consistent IP policy will assist the Bean/Cowpea CRSP in meeting federal obligations and provide clear guidance on the management of intellectual property rights within the CRSP.
- ◆ **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 Indonesia (7), Costa Rica (1), South Africa (1), Egypt (4), Morocco (5), and Kenya (2) to attend the annual or regional meetings of AUTM in the US.

To assist in the marketing of intellectual properties, the ABSP project assisted developing countries in setting up booths at the AUTM technology transfer fair in 2002 at the annual meeting of AUTM in San Diego. ABSP has sponsored participation of several individuals from Indonesia and Egypt at these courses. ABSP Partner country participants were thus exposed to the larger US University community and technology transfer practices and could actively network with meeting participants. .

◆ **Support to Technology Transfer Office, ARC Egypt, 2001**

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

◆ **Egyptian IPR and PVP Training, at MSU September 2001**

As a result of the above consultation in Egypt for the ARC (Ministry of Agriculture), Drs. Erbisch and Karim Maredia, with ABSP staff support, developed and participated in two additional short-term training programs at Michigan State University in September 2001. One program was on intellectual property management and was presented to 9 representatives from the Egyptian ARC. The 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. The ARC is now in the process of establishing a technology transfer office in Egypt that will be operated by participants in the September workshop.

◆ **IPR/Legal Clearances for Potatoes—Obtaining Freedom to Operate (FTO) and Establishing Licensing Relationships**

The ABSP Project at Michigan State University (MSU) licensed the *cry11a1* gene from the ICI Seeds Company on October 21, 1994. The gene was licensed for research purposes only to develop transgenic potatoes resistant to the potato tuber moth (PTM). The initial license was for three years and was extended for an additional 3 years (until October 5, 2000) by the Garst Seed Company on October 7, 1997. Syngenta Company provided a further extension until June 2002, and this was then extended until December 31, 2002.

The ABSP project conducted an initial intellectual property (IP) audit of the Bt potato lines and based on this IP audit, determined the following third party intellectual property used:

1. Spunta variety (now in the public domain)
2. *Cry11A1* Gene (Syngenta Company)
3. Nos-Npt-II selectable marker (Monsanto Company)
4. CAMV 35s promoter (Monsanto Company)
5. *Agrobacterium* transformation system (Monsanto Company and others)
6. Binary Vector system (formerly MOGEN, now Syngenta Company)
7. Codon modification for expression of Bt genes (Monsanto Company)
8. PBI121 plasmid (John Innes Institute?)

In January 2003, an ABSP delegation, along with USAID met with Syngenta representatives in Basel, Switzerland to discuss the commercialization of Bt potatoes in developing countries. The Syngenta-company is willing to grant a royalty free license to ARC for the use of *Cry11A1* gene and to work with MSU to obtain freedom to operate (FTO) on third party intellectual properties used in the development of the Bt potato product. The MSU Bt Potato team will work with the MSU-Office of Intellectual Property (OIP), Syngenta, other companies and other appropriate parties in partner countries to refine the findings of the initial audit, and to ensure that all the rights necessary to commercialize this technology are obtained.

◆ **IPR/Legal Clearances for High Vitamin A Mustard Oil, 2001-2003**

ABSP has been working with all the partners involved in this collaboration to ensure freedom to operate for any varieties of mustard with enhanced vitamin A content developed from this project. The current research agreement between TERI and Monsanto expires in 2005. TERI and MSU are committed to continue this project to develop, commercialize and deliver a final product. A preliminary inventory of the intellectual properties (IPs) involved in this project identified the following:

- ◆ Mustard Varieties: Pusa Bold, Varuna, and YSRL; Pusa Bold is a national check variety developed by IARI. Transformation is currently in progress at TERI with the YSRL variety.
- ◆ Genes: Psy, Crt 1, NPT-II
- ◆ Promoter: Napin

In order to commercialize this technology in India, an IPR Freedom to Operate (FTO) and commercialization agreement needs to be negotiated and finalized with Monsanto. This process is currently underway.

◆ **ABSP Technology Transfer Workbook, 2002-2003**

ABSP's Intellectual Property Consultant, Dr. Fred Erbsich, authored a training manual based on the experience gained during the six years of running the IP and Technology Transfer Course at MSU: BasicWorkbook In Intellectual Property Management. The workbook is intended to be a ready source for continued training and reference for participants on the MSU course, and will be a useful stand-alone training document.

The objective of this book is to provide basic information regarding the handling of intellectual properties; to answer questions concerning the types of agreements used transferring intellectual properties from one organization to another or one researcher to another researcher; to provide awareness as to the important sections of agreements and why these sections are important; and to provide a base for organizations in various countries to develop "standard" intellectual property transfer agreements that are in accordance with the country's laws as well as the organization's policies. Each chapter of the book is complete in itself and there is no need to refer to another chapter for additional information. Most chapters have a homework section where the reader is asked to modify the sample agreements.

The workbook was reviewed by an international panel of reviewers, and a near final draft of the workbook was completed in early 2003. This version is now undergoing final revision and formatting to be made available for download from the ABSP website by November 2003.

◆ **USAID Biotechnology Program Development in Africa, 2001-2002**

In 2001/2002 USAID commissioned ABSP to manage several Biotechnology Assessments in South Africa, Uganda and Kenya. The overall purpose of these assessments was to assist the local USAID missions to assess the current status of agricultural biotechnology in the countries, and with this information to design a bilateral biotechnology program which would support Mission strategies, address national agricultural development priorities, and take advantage of ongoing biotechnology research in the international community. The assessment reports were well received by USAID and were used by the Missions and USAID/Washington to help frame their decisions for continuing support to the utilization and implementation of biotechnology as a tool in the fight to cut hunger in Africa.

◆ **Grades & Standards Assessments**

In 2000/2001 ABSP sponsored four assessments on the need for and importance of grades and standards (G&S) for select commodities and countries in Africa. In 2000 the

first assessment was focused on fruits and vegetables in Kenya and the second assessment focused on a number of commodities in Malawi. In 2001 similar assessments were carried out in Mozambique and Zambia.

The Zambia study assessed whether more effective and efficient systems of Grades and Standards (G&S) could improve Zambia's agricultural sector and expand its trade and development opportunities. Overall the study concluded that G&S is a necessary but not sufficient factor to dramatically improve Zambia's competitive position. However, the report suggested that if Zambia undertakes to better understand and make use of G&S, it could utilize these strategic tools for product differentiation, market penetration, and system coordination, as well as quality and safety assurance.

The Mozambique assessment team found that G&S are not generally perceived to be a high priority in Mozambique in the face of other constraints to production and marketing of agricultural commodities such as physical infrastructure, institutional infrastructure and low productivity. In spite of the current lack of a significant national/regional momentum on G&S issues in Mozambique, the team revealed the strategic significance of G&S within specific sub sectors in promoting agricultural/fishery export growth. A range of G&S issues impact significantly on these sub sectors as they are predicted to with non-traditional commodities such as pigeon pea, sesame, banana, citrus, and paprika, which are all regarded as having export potential in the country.

These assessments are now being used to direct the research and development work of the USAID funded Partnerships for Food Industry Development (PFID) program based at Michigan State University

◆ Socioeconomic Analyses

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

Dr. Molly Jahn at Cornell University has been highly successful in building linkages with private seed companies, in the U.S. and in several developing countries, for field-testing and commercialization of the multiple resistant cucurbit germplasm developed under ABSP. In 2001 the ABSP supported socioeconomic assessments of virus-resistant cucurbits in Indonesia and South Africa. The purpose of these assessments was to determine the potential benefit of virus disease resistant cultivars to farmers, consumers and seed companies in these two developing countries. Private seed companies in Indonesia and South Africa had already received multiple virus resistant cucurbit germplasm from Cornell prior to the socioeconomic assessment.

In each country the assessment was carried out by a multidisciplinary team, which included a socio-economist, a cucurbit breeder and the ABSP technology transfer coordinator. These assessments provided several key insights with respect to the characteristics of the cucurbit sub sector in Indonesia and South Africa. Recent field tests of the Cornell materials demonstrate that their sources of resistance appear to be valuable in the Indonesian and South African growing environments. Under various cost-benefit analysis scenarios the rate of return to ABSP investment in the Cornell cucurbits program is positive. The studies concluded that keys to increasing future ABSP impact in cucurbits include: performing socioeconomic assessment of cucurbit sub sectors in target countries to help set collaborative breeding priorities; encouraging Cornell to collaborate with multiple firms in each country, in order to avoid monopoly pricing of new technologies.

Economic Analysis Of Genetically Modified Potatoes in South Africa and Egypt

ABSP supported a team from the University of Idaho to carry out an economic analysis of the planned commercial release in South Africa of the potato cultivars developed at MSU to be resistant to the potato tuber moth (PTM). A similar assessment in Egypt was also carried out in Egypt. The objectives of this studies were to describe the potato industries, to estimate the economic value of PTM-resistance and to analyze pertinent biotechnology

issues in both countries. An ex-ante impact assessment was used to first evaluate the potential impact on the individual farm level, followed by an analysis of the market level effects. The PTM-resistant technology was found to contribute significant benefits at the farm level in terms of reduced insecticide costs, increased yields, improved quality and reduced post-harvest losses. The model used also indicated that consumer benefits—including larger supplies, lower prices and better quality potatoes—would be larger than producer benefits. According to the study, consumer acceptance is not expected to be a major barrier to development in either country.

The PTM-resistant technology that benefits producers and consumers in South Africa could also be beneficial in other countries in the region. Egyptian potato experts familiar with PTM spread suggest that the technology would be beneficial to all Middle East countries and the African countries on the Mediterranean Sea. In addition, many Sub-Saharan African countries, including Uganda and Kenya also have PTM problems. This analysis based on only two countries could therefore be a very conservative estimate of total benefits that would accrue in the region. If the PTM-technology does not become available in other countries this study may have underestimated total benefits because international trade was not included in the analysis. Both Egypt and South Africa are players in the global potato market, and this technology has the ability to significantly increase potato exports. As the marketable yield and quality increase, the price of the product will fall, providing incentive to export to other countries at a competitive price, and South Africa already has a competitive advantage in its ability to harvest potatoes year round.

Valuable information was collected in Egypt and South Africa that will assist the development of a roadmap for commercialization and dissemination of the tuber moth resistant potatoes during the next few years.

Impacts

Indonesia

◆ Establishment of Technology Transfer Office

Through training and technical assistance from ABSP, the Agency for Agricultural Research and Development (AARD; equivalent to the Agricultural Research Service/USDA) established a new office of Intellectual Property and Technology Transfer in Bogor, Indonesia, July 1999. The ABSP office trained two staff members in various issues of IP management and technology transfer. The office (known by the Indonesian acronym KIAT) is now actively involved in educating scientists and policy makers in Indonesia in management of IP. KIAT is also working with the private sector to license technologies generated within AARD institution and will serve as the main focal point for management of intellectual properties related to agriculture/biotechnology. The AARD is one of the few developing country institutions to recognize the benefits of intellectual property and to develop within the ministry a system for protecting and exploiting Indonesian innovations to benefit Indonesian agriculture. Within 3 months of its operation, KIAT had executed 5 license agreements to commercialize a wide range of technologies developed by the AARD institutions.

According to Dr. Achmad Fagi, Secretary General of the AARD, this office is the direct result of training received in IPR and Technology Transfer at MSU via the short course. The office will have a legal and financial division, general business division, technical division, and a secretariat. Ketty Karyati, who has received training as part of ABSP's capacity building efforts with Indonesia, will be the administrator of the office as the secretary. KIAT has expressed interest in running an in-country IPR workshop to educate

key scientists and various AARD institutions. In addition, MSU's draft IP policy was shared with KIAT to be used as a basis for developing a system-wide policy in IP.

KIAT recently changed its name to the Intellectual Property and Technology Transfer Office (IPTTO), still under the Agency for Agricultural Research and Development (AARD). To assist IPTTO, a Technology Commercial Unit has now been formed in each AARD Research Institute.

Since it was established, KIAT has licensed 43 plant varieties, using trademark licensing and technologies such as biofertilizer and biopesticides. Most recently they have licensed two rice hybrids varieties. It is hoped that the office will be self sufficient in 2004.

◆ **Founding of the Indonesian Inventor Society.**

Dr. Didiék Hadjar from the Estate Crops Research Institute attended the MSU IPR and Technology Transfer Course in 1998. He has since co-founded a new organization called the Indonesian Inventor Society and is serving as President. Again, this organization was developed as a direct result of Dr. Didiék's participation in the course. There have been several biofertilizer/biofungicide technologies patented with the assistance of this organization and are in various stages of commercialization.

◆ **Indonesian PVP Law passed in 2001.**

In December 2000, the Indonesian Parliament approved the Plant Variety Protection (PVP) Act. This law is based on the UPOV 1991 Convention. ABSP assisted in drafting this new PVP law in 1995, and researchers trained by ABSP have been working with the Minister of Agriculture to educate the Parliament about the law. In order to implement the Plant Variety Protection Law that was stated in 2002, the Center for Plant Variety Protection was established. This Center has drafted the two Government Regulations and 4 Ministerial Decrees.

Egypt

◆ **Establishment of the Technology Transfer Office at Agricultural Genetic Engineering Research Institute (AGERI).**

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 a 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).**

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.**

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.

Philippines

◆ **Drafting of Plant Variety Protection Legislation.**

Ms. Conception Magboo from the Philippines attended the IPR Internship Program at MSU during the summer of 1999. Ms. Magboo is now a member of the team that is drafting the Plant Variety Protection Act in the Philippines. Her participation in the IPR internship program was sponsored by ISNAR.

Morocco

◆ **Plant Variety Protection Law.**

In December 1996 the Moroccan Parliament passed legislation for the protection of new and improved plant varieties. The new law, Number 9-94, conforms to the 1991 International Union for the Protection of New Varieties of Plants (UPOV) convention. In 2000, the ABSP learned that Morocco has been accepted into UPOV. The DPVCTRF has a well-equipped office with trained scientists to implement the Moroccan PVP law.

Kenya

Kenya has also received support in IPR and Technology Management, primarily through support of Kenyan scientists to the MSU IPR course. Impacts include:

- ◆ Development of trained staff within the Kenyan Plant Breeders' Rights Registration Office (PBRR).
- ◆ The Plant Breeder's Rights Registration Office (PBRO) was established in 1997 and has received over 300 applications, of which 15 have been provisionally granted (as of 2002). Three-quarters of the applications have been on cut flowers, but others have been on local varieties of crops.

ABSP Networking Activities

From the outset of the project, the ABSP was designed to build and strengthen national agricultural systems, to deliver a specific set of research products and information packages, and to develop genuine bridges of collaboration between the US and developing country partners. One of the project's initial objectives was to build a global network that provided access to information and serves as a forum for the exchange of ideas and information on biotechnology in relation to sustainable agriculture systems. Within ABSP's networking activities, the focus has been on the development of newsletters, implementation of workshops, linkage of developing country partners to the biotechnology industry, and the development of other electronic and print media.

Membership in BIO

To facilitate interactions between the ABSP collaborating countries and the private sector in the US, the ABSP project provides memberships in the Biotechnology Industry Organization (BIO) to partner countries. The memberships help provide new information and build linkages between the public and private sectors in developed and developing countries. Membership in BIO provides access to member institutions, a newsletter which highlights research innovations and policy issues, and lowers fees for attending the annual BIO meeting and exposition. ABSP also supported the participation of individuals from partner countries in annual BIO meetings and organized sessions highlighting the research contributions and economic opportunities in partner countries.

ABSP Industrial Seminar Series

In April 1993, the ABSP organized an Industrial Seminar Series (ISS). The ISS was organized to provide opportunities for senior scientists and administrators from the public and private sector, and government officials from the ABSP partner countries (Costa Rica, Egypt, Indonesia, Kenya) to interact with technical and business personnel at private biotechnology companies in the U.S. that have active agricultural biotechnology programs. In addition to seven participants from the ABSP partner countries, three participants from Jamaica (sponsored by the USAID-Jamaica) also attended the ISS. The companies visited included Garst/ICI Seeds, Inc. (now Syngenta), Ecogen Inc (now part of Monsanto), and DNA Plant Technology.

The ISS was instrumental in opening lines of communication between developing country leaders and host companies. It also provided participants an exposure to a diverse group of companies oriented towards different end-user groups.

Other impacts of the ISS included:

- ◆ A Memorandum of Understanding (MOU) was developed between Agriobiotechnologia de Costa Rica and Fitotek Unggul (Indonesia) to collaborate in tissue culture and micropropagation of bananas and other horticultural crops.

Biolink

In the first phase of ABSP a quarterly newsletter, *BioLink*, was distributed free-of-charge to 2,500 individuals and institutions in 115 countries. Electronic copies of some of the back editions of *BioLink* are still available from the ABSP website (<http://www.iaa.msu.edu/absp/publications.html>). This publication was extremely well received and received numerous awards for design.

- ◆ **BioLink Volume 1, Number 1**, Cover story: The ABSP Idea: International Agricultural Research and Development
- ◆ **BioLink Volume 1, Number 2**, Cover story: Indonesia's Foundation in Biotechnology

- ◆ **BioLink Volume 1, Number 3**, Cover story: Genetic Engineering - Addressing Agricultural Research Needs in Egypt
- ◆ **BioLink Volume 1, Number 4**, Cover story: Intellectual Property Rights: The ABSP IPR/Patent Workshop
- ◆ **BioLink Volume 2, Number 1**, Cover story: Fitotek Investigates the Plant Bioreactor
- ◆ **BioLink Volume 2, Number 2 and 3**, Cover story: ABSP Enters Its Exciting Technology Transfer Phase

In view of the increased availability of electronic communications, and to reduce publication and postage costs, in Phase II of the project we made the decision to distribute our newsletter, (*Linkages*) by email (see next section).

LINKAGES Newsletter

The first electronic *ABSP LINKAGES* newsletter was distributed in April of 1999 to about 350 contacts. The newsletter is sent quarterly by electronic mail and includes commentary from the ABSP Director, a feature article, and reports from ABSP domestic and international sources on current events and travel in the past quarter. *LINKAGES* focuses on cooperating country activities, accomplishments and events for the past quarter as well as a brief overview of upcoming events and projects. The 3rd quarter 2002 *LINKAGES* was distributed to over 900 contacts, almost triple the size of the first electronic newsletter distribution. Contact email addresses were exported from the ABSP database for each newsletter mailing, which helped to keep the distribution of the newsletter as current as possible. *LINKAGES* is also posted to the ABSP web site under ABSP News (<http://www.iaa.msu.edu/absp/news.html>). *LINKAGES* was distributed quarterly throughout Phase II of the project to over 800 individuals and institutions worldwide. Feedback and responses from the newsletter to ABSP have been very positive.

CABI & AgbiotechNet

Although not available at the outset of the ABSP, there are currently numerous listservs and news sites on agricultural biotechnology. *AgBiotechNet*, published by CABI, provides current information about biotechnology and biosafety for researchers and policy makers worldwide. The site gives rapid and convenient access to research developments in genetic engineering and updates on economic and social issues. The contents and user community of *AgBiotechNet* continued to grow since launch in January 1999, and evolved considerably in design to include novel ways of tailoring it to meet the needs of different users. The average number of user sessions per day since the launch in January 1999 increased from around 80 in January 1999 to over 300 in December 2000. In 2001 *AgBiotechNet* had 447 subscriber registrations from institutions and individuals, over 1,600,759 successful hits on the site, approximately 2,800 per day. There were 2984 direct *click-throughs* from *AgBiotechNet* to ABSP's website, according to the hit counter on the *AgBiotechNet* site.

AgBiotechNet hosts a popular information section on biotechnology and developing countries. A 'hot topic' on the subject, incorporating news, reviews, abstracts, and structured links is one of the most frequently visited pages on *AgBiotechNet*. CABI *Publishing* continues to work with a wide range of international development and research organizations generating content in the field, and *AgBiotechNet* contains many of the popular ISAAA *Brief* documents, a series of articles commissioned by IFPRI, and the most recent reports from the National Agricultural Biotechnology Council. By 2000 there were over 80,000 records available in the abstracts database, with around 1300 added per month.

ABSP supported the membership of the following developing country institutions to *AgBiotechNet*:

- ◆ Zamarano Escuela Agricola Panamericana, Honduras.
- ◆ Kenya Agricultural Research Institute, Kenya.

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

Global Conference

The ABSP Global Conference, *Agricultural Biotechnology for a Better World*, was held April 28-30, 1997. Held at the Asilomar Conference Center in Pacific Grove, California, the conference was attended by approximately 300 people, with participants from 25 countries, 30 private companies and 50 research institutions. The conference integrated a number of essential components including technological development, regulatory requirements, technology transfer and recognition of commercialization requirements. Potential mechanisms for collaborations to address mutual needs and interests were discussed in formal and informal settings. CABI published the proceedings as a component to their *Biotechnology in Agriculture Series*, with the volume entitled *Agricultural Biotechnology in International Development* (ISBN 0 85199 278 1).

ABSP Database Development

In late 1997, it was determined that communication and office management would be better served with the creation of an ABSP database of contact information for electronic and postal communications, travel information, conference and workshop management and other information such as areas of expertise for ABSP contacts. The Management Team has continued to expand and develop the contacts and upgrade and improve the database management system. In 2000 we included an improved contact database of expertise in all fields of agricultural biotechnology. The database currently contains over 1,000 contacts and has proved to be an extremely valuable resource for the project.

ABSP Website

The ABSP Website was initially developed from a one page site in 1996 to become a popular and valuable resource in agricultural biotechnology. It has been continuously updated and upgraded since this time, and continues to receive an increasing amount of traffic. There have been over 32,000 visitors to the Website since August of 1999, with the average monthly number rising to a high of over 1,000 in November 2001. This coincides with a significant updating of the web pages, and the addition of a 'Links' page, giving links to other web sites on agricultural biotechnology and developing country issues. Other peaks in visits coincided with the posting of the Agricultural Biotechnology Inventory for Africa on the web pages (over 1,300 copies downloaded), the availability for download of *Biosafety & Risk Assessment in Agricultural Biotechnology: A Workbook for Technical Training* (over 1,000 downloads), and also the submission of the web site details to a range of internet search engines.

Most of the major agricultural biotechnology websites worldwide now have a link to the ABSP site from their pages—a total of 325 links to the site recorded as of September 2003. The most requested pages (not including the home page) were the News (29%) pages where ABSP's newsletter Linkages is posted, followed by the page with information and downloads of ABSP's Biosafety Workbook (22%), Research pages (19%), Links to other sites on agricultural biotechnology (8%), Technology Transfer (7%), background (8%) and Policy Issues (7%).

Information on MSU's summer short courses in Intellectual Property Rights, Food Safety and Integrated Pest Management were also placed on the web site, with a new online registration enquiry form (<http://www.iaa.msu.edu/absp/msucourses03.html>). This method of advertising the courses proved very effective, with over 60 applications to the courses submitted directly from the website.

The ABSP website has won two major awards for its design and content; in June 1999 the site was awarded HMS Beagle's (<http://news.bmn.com/hmsbeagle>) "*Web Pick of the Day*"; and in February 2000 it was selected in Lightspan's StudyWeb® (<http://www.studyweb.com/>) as one of the best educational resources on the Web.

Electronic Listserv on Biotechnology for USAID

In 1999 ABSP and the USAID biotechnology officer co-developed an electronic email Listserv to engage USAID staff based in the US and in overseas missions in a broader discussion of agricultural biotechnology. The aim of the Listserv was to increase awareness of the issues and opportunities surrounding biotechnology and to explore possible new programmatic approaches on a national, regional and global basis. The listserv was targeted to an audience of government officials and agencies, including 45 USAID employees or contractors. The USAID–Biotechforum Listserv postings were issued bi-weekly from April – July 2000. By popular request these postings have now been placed on the ABSP website as a *Biotechnology Primer* (<http://www.iaa.msu.edu/absp/biotech1.html>) and will also soon be published by USAID's Bureau for Africa.

Commercialization Study of AGERI, Egypt

A three-year project assessment of AGERU, Egypt 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, involving interviews with the biotechnology industry and the agriculture sector, and an analysis of possible demand for AGERI projects with estimates of the resulting revenue streams. Following completion of the assessment, the team made recommendations to AGERI which included the re-evaluation of the business strategy and clarification of the organizational mission with a view to developing a more strategic marketing plan.
- ◆ **Strategic Marketing Plan for AGERI, 2000:** The second Haas team in 2000 developed a strategic marketing plan for AGERI, looking at the agricultural biotechnology sector and at issues relating to genetic engineering of food crops. Some over-arching recommendations made by the team for AGERI included advancing the institutes market-driven philosophy, expanding its management capability and evaluating projects based on a more market-based metric.
- ◆ **Preparing AGERI for Continued Success in the Evolving Biotechnology Industry, 2001:** The third Haas team investigated prospects related to AGERI's internal organization, and recommended some crucial management changes such the further development of their mission, vision and values statements, a more rigorous market-focus to guide project selection, and to proactively pursue an endowment funded by USAID.

ABSP Egypt Project Symposium

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

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.

Bread for the World Institute Workshop

ABSP was a co-sponsor for the Bread for the World Institute (BFWI) Conference on Agricultural Biotechnology - Can it help reduce Hunger in Africa, held in Washington DC from March 5-7, 2002. The conference was organized by BFWI through a grant from the Rockefeller Foundation and with support from ABSP. ABSP funding covered the travel and expenses of 10 African delegates. The conference explored the question "*Can agricultural biotechnology address hunger concerns in Africa?*" Over 100 participants attended from local and international NGOs, universities, government and international agencies and institutions in Africa. Case studies from Africa were presented and provided an opportunity to discuss important issues raised by the speakers and panels. Among the 23 conference presentations were luncheon speeches by Ambassador Edith Ssempera of Uganda and Peter McPherson, President of Michigan State University, and a closing session speech by U.S. Undersecretary of State Alan Larson.

ABSP worked closely with the BFWI to develop the program for the meeting, to select speakers, and in the identification of the appropriate African participants to invite. The presence of the African delegates was a vital factor in the success of the meeting as it provided a much-needed opportunity for the voices of Africans to be heard in the biotechnology debate. African delegates were able to network extensively with US policy makers, leaders from the donor community, NGOs and scientists.

Evaluation reports, both verbal and written, indicate that the conference met its objectives, assembled diverse voices and allowed discussion on biotechnology as it relates to hunger in Africa, as well as helped attendees establish new contacts and share their concerns about biotechnology. Many of the respondents expressed the need for similar meetings in the future.

- ◆ African delegates were provided a much-needed opportunity for their own voices to be heard in the biotechnology debate. They networked extensively with US policy makers, leaders from the donor community, NGOs and scientists.
- ◆ A publication of the proceedings was made available in early 2003.
- ◆ The conference was used as a springboard for a draft biotech policy for BFW. This was shared with BFW's Board Members, and the organization's faith-based partners.

FAO Biotechnology Symposium

ABSP co-sponsored a plant biotechnology symposium *Perspectives from Developing Countries: Towards a Global Strategy, Partnership and Action Plan for Food Security and Poverty Alleviation* organized by the Food and Agriculture Organization (FAO) and the Crop Science Society of America. This symposium was held during the Annual Meeting of the American Society of Agronomy, the Soil Science Society of America and the Crop Science Society of America in Indianapolis, Indiana (12-14 November 2002). ABSP funding covered the travel and expenses of 20 developing country participants.

- ◆ The objectives of the meeting were:
- ◆ To present policy, regulatory and research status of agricultural biotechnology from developing countries;
- ◆ To present biotechnology initiatives by international stakeholders such as the CGIAR, FAO, OECD, UNDP, WIPO, World Bank, USAID; and
- ◆ To discuss modalities to strengthen coherence of global partnerships integrating biotechnology tools for food security and poverty alleviation in developing countries.

As a result of the meeting a briefing note was produced containing a summary of the technical experts' consultation together with framework for global plant biotechnology strategy, global partnerships and an action plan. A proceeding of the experts' presentation was also produced, including a plant biotechnology strategy for global partnerships and an indicative action plan to harness the power of biotechnology through multi-stakeholder cooperation for the benefit of developing countries.

ABSP Publications

ABSP Publications

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Final Technical Reports

Potato Transformation and Field Testing for the Development of Potato Tuber Moth Resistance—Final Report

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Overall Project Goal

High dose expression of *Bacillus thuringiensis* (Bt) in potato plants offers an ecologically sound means to control the potato tuber moth. Our goal is to develop and sustain genetically engineered host plant resistance factors in potato to control the potato tuber moth. Transgenic potatoes will reduce insecticide use thus allowing for the production of a safer product for human consumption. Additionally, potato production with less insecticide use will have less impact on the insecticide contamination of the environment.

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.

Rational for Approach

The Bt-cry1Ia1 Bt toxin gene has been codon modified to increase its expression level in the plant and transferred into potato. Douches et al. (1998) used different Bt-cry1Ia1 constructs for engineering the potato to express high level of Bt toxin effective against potato tuber moth. Some of these transgenic lines were evaluated to determine the foliar resistance to potato tuber moth (Westedt et al. 1998). Li et al. (1999) produced a series of Bt-cry1Ia1-Bt and transformed the cultivar Spunta. Foliage bioassays with potato tuber moth revealed high expression levels. For these transgenic lines to be commercially successful in reducing potato tuber moth damage, we must assess the tuber resistance from the field and storage. Our approach was to evaluate field-grown Bt-cry1Ia1-Bt transgenic potato tubers for their resistance to potato tuber moth in the field and in ambient temperature storage facilities.

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

Previous Potato Project Research

Initially, transformations with the *cryIa(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-cry1Ia1* construct (with the *gus* gene fused to the *Bt-cry1Ia1* 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-cry1Ia1* 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-cry1Ia1-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-cryI* and *Bt-cry1Ia1* 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-cryIa*-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-cry1Ia1*-Spunta and *Bt-cry1Ia1/PVY*-Spunta with high levels of potato tuber moth mortality (Li et al 1999). Other *Bt-cry1Ia1*-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-cry11a1* 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-cry11a1* 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-cry11a1* 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 non-transformed Spunta lines also had little damage. 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.

Potato Project Specific Objectives

- ◆ Genetically engineer potato varieties important to Egypt and South Africa 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, AGERI and ARC-Roodeplaat) and field tests in Egypt and South Africa.
- ◆ Evaluate the effectiveness of tubers stored in ambient temperature storage structures in controlling potato tuber moth.
- ◆ Evaluate the efficacy of transformed potatoes in controlling other Lepidopteran potato pests.
- ◆ Evaluate the effectiveness of individual and combined resistance factors under laboratory experiments.
- ◆ Train scientists from Egypt and South Africa 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 and South African seed companies to promote the commercialization of desired potato lines.
- ◆ Evaluate different management strategies for maintenance of resistant potato varieties and their integration into IPM systems.

Research Progress

Two field trials were planted in South Africa in 2002. One field trial was established at Roodeplaat and the other at Ceres. Both trials were harvested in 2003. Additionally, a storage trial was conducted at Roodeplaat. Six potato lines containing the *Bt-Cry11a1* gene and two non-GMO controls, Spunta and BP1 were used in all trials. The field trial at Ceres showed complete resistance with no damage to the foliage of any transformed line. The non-transformed controls were severely attacked. Despite field releases of more than 30 000 tuber moths, the trial at Roodeplaat showed a near zero infestation (even in the controls) and the efficacy of the transformed lines could not be evaluated. The storage trial in a diffused light store was extended from last year's trial. All transformed lines (except S4) provided total control against potato tuber moth for at least eight months in open storage. The S4 line showed minimal damage from tuberworm.

Laboratory studies were conducted at MSU to evaluate the efficacy of transformed potatoes in controlling other potato insect pests. Five lepidopteran species (European corn borer, cabbage looper black cutworm, beet armyworm and tomato hornworm) were fed foliage from G2, G3, 6a3 and non-transformed Spunta plants and insect mortality was recorded after 72 hours. The black cutworms were also tested against tubers of the same lines. The transformed lines provide excellent control of European corn borer and tomato hornworm and a low level of control of black cutworm and beet armyworm compared to the control. No control of cabbage loopers was observed with the transgenic lines.

Studies were also conducted to evaluate the efficacy of transformed plants with different promoters (ubiquitin, super promoter and 35s) in controlling Colorado potato beetle larva. All of the transformed lines provided some control of neonate beetle larvae compared to non-transformed controls. However, several of the lines with the ubiquitin promoter gave total control.

Seed increases of transformed Atlantic and Lady Rosetta lines were made at MSU and their agronomic properties were evaluated. No differences were found between the transformed and control tubers with respect to yield weight, size and internal qualities.

MSU is collecting data necessary for commercial approval of Spunta G2 and G3 potato lines in South Africa and Egypt, and other countries where this transgenic line will be useful. Listed below is the data collected thus far that are relevant to food and environmental safety assessments.

Existing Data

A. Molecular Characterization

1. Description of construction and map of the vector, pSPUD5

The basal skeleton of both constructs in this experiment was the binary vector pBIN19 (Bevan, 1984). A Bluescript (Stratagene, CA) plasmid harboring a codon-modified *cry1Ia* gene, was supplied by ICI Seed/Zeneca (Berkshire, UK). The *cry1Ia* gene was cut from the Bluescript plasmid and inserted into the *BamHI* site of pBI121. This plasmid was called pSPUD2. The *gus* gene was then removed by digesting pSPUD2 plasmid with *SmaI* and *EcoICRI* (Statagene). The resulting blunt ended fragment was religated creating CaMV35S plasmid called pSPUD5 (Fig.1). pSPUD5 was mobilized into *A. tumefaciens* LBA4404 from *E. coli* by triparental mating (Bevan et al., 1984).

2. Donor genes and regulatory sequences

- a) The *cry1Ia1* gene (previously *cry V*), and its encoded cry 5 protein

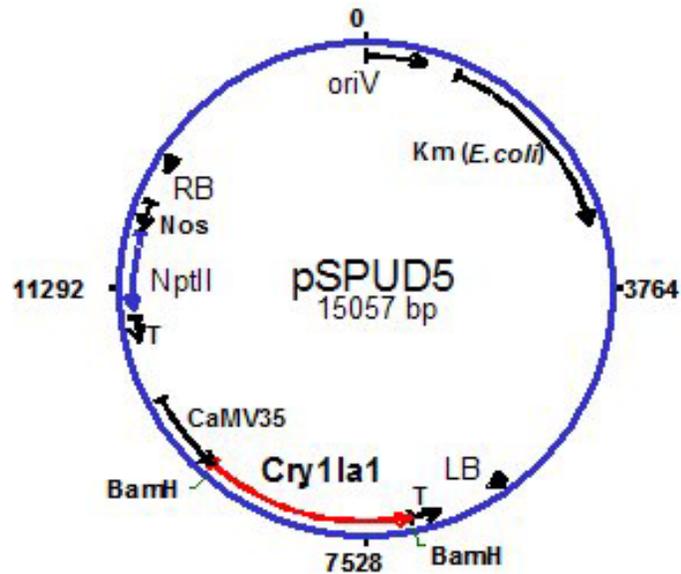


Figure 1 pSPUD5 the *Bt-cry1Ia1* vector construct used in *Agrobacterium tumefaciens* transformations with Spunta.

The *cry1Ia1* gene was described by Tailor et al. (1992). The gene was codon modified by Garst Seeds, Slater, Iowa (now a subsidiary of Syngenta Seeds).

- b) The *nptII* gene and its encoded nptII proteinThe *nptII* gene was donated by the transformation vector pBI121 (Bevan, 1984). The complete nucleotide sequence of this vector is deposited in GenBank (accession number AF485783, nucleotides 2383-3632 comprise *nptII*). *NptII* encodes the neomycin phosphotransferase II protein. The safety of *nptII* well established (Fuchs et al., 1992 a, b; Flavell et al., 1992; Nap et al., 1992; Betz et al., 2000)

3. Genetic Analysis

- a) Characterization of inserted DNA

1. copy number

Unpublished Southern hybridization of Spunta lines digested with *Xba*I and probed with a *cry1Ia1* probe are available. Spunta G2 appears to have only one copy, while Spunta G3 has three copies.

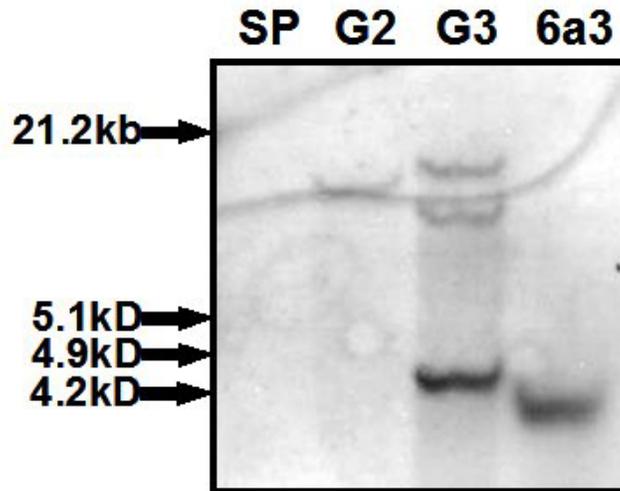


Figure 2. Southern hybridization of Spunta lines digested with Xba I for copy number and probed with a DNA *Bt-cry1IA1* DIG probe.

2. intactness of inserted genes

Li et al. (1999), report that a Southern blot of genomic DNA from Spunta G3, digested with *Bam*HI/*Sac*I and probed with the *cry1la1* gene shows the expected 2.2 kb.

Other unpublished results (Figure 3 and Figure 4) using PCR and Southern analysis indicate an intact *cry1la1* gene. The PCR amplification of the *nptII* gene indicates the expected size, indicating intactness of the gene between the primer sequences.

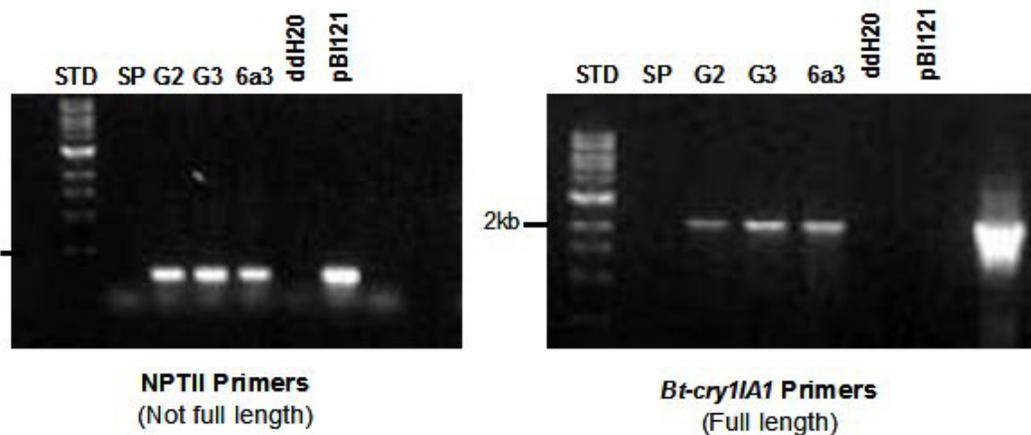


Figure 3. PCR amplification of the *nptII* gene and the *cry1la1* gene from Spunta G2 and Spunta G3.

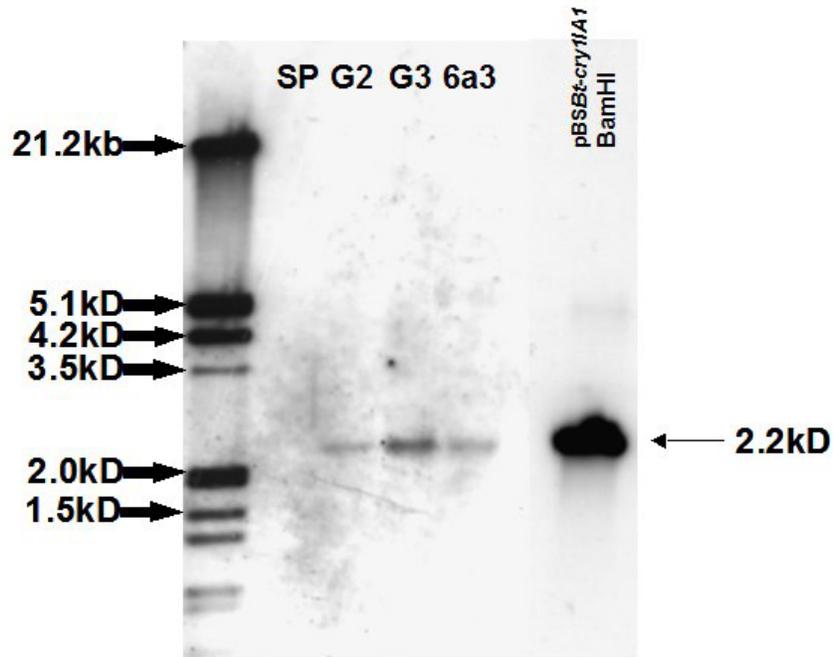


Figure 4. Southern hybridization of Spunta lines digested with BamHI and probed with a DNA Cry1Ia1 DIG probe to confirm intactness of the *cry1Ia1* gene.

3. presence/absence of backbone sequences

PCR and Southern analysis was done to determine presence of the vector backbone in Spunta G2 and Spunta G3. The results indicate backbone is present in Spunta G3.

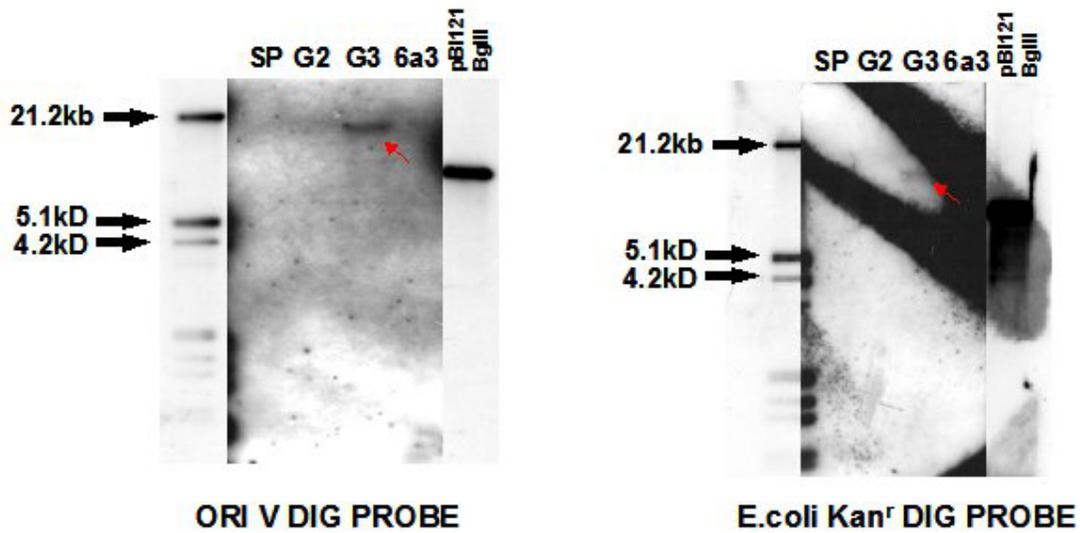


Figure 5. Southern analysis showing the absence of the bacterial ori V and kanamycin resistance genes in Spunta G2, but presence in Spunta G3.

b) Characterization of expressed proteins

All data to date were obtained from greenhouse-grown plants.

1. cry1Ia1

(a) Expression level—leaf

Northern analysis of Spunta G2 and Spunta G3 leaf tissue is reported in Li et al. (1999). Unpublished northern data are also available. They show the expected transcript size.

Unpublished results confirm expression of RNA in Spunta G2 and Spunta G3.

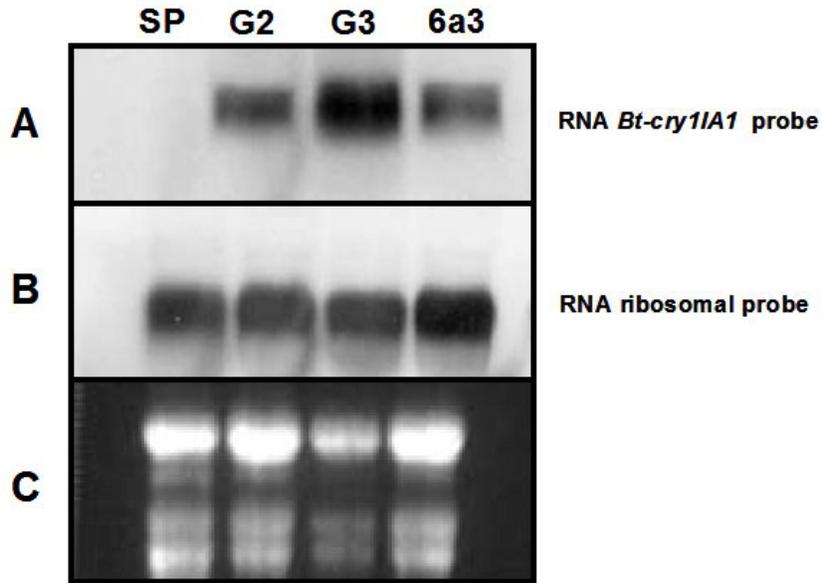


Figure 6. RNA analysis of Spunta lines. Panel A is a northern hybridization using a DIG labeled **RNA *Bt-cry1IA1* probe**. Panel B is the same blot used in Panel A but stripped and re-probed with a DIG labeled **RNA ribosomal probe**. Panel C is the Ethidium bromide stained gel used in the transfer to the blot.

Western analysis from leaf tissue is reported in Li et al. (1999). No quantitative expression levels are reported. Recent unpublished western analysis (Figure 7) indicates that cry1a1 protein levels are 1.0 µg/mg tissue and 2.8 µg/mg tissue in Spunta G2 and Spunta G3, respectively.

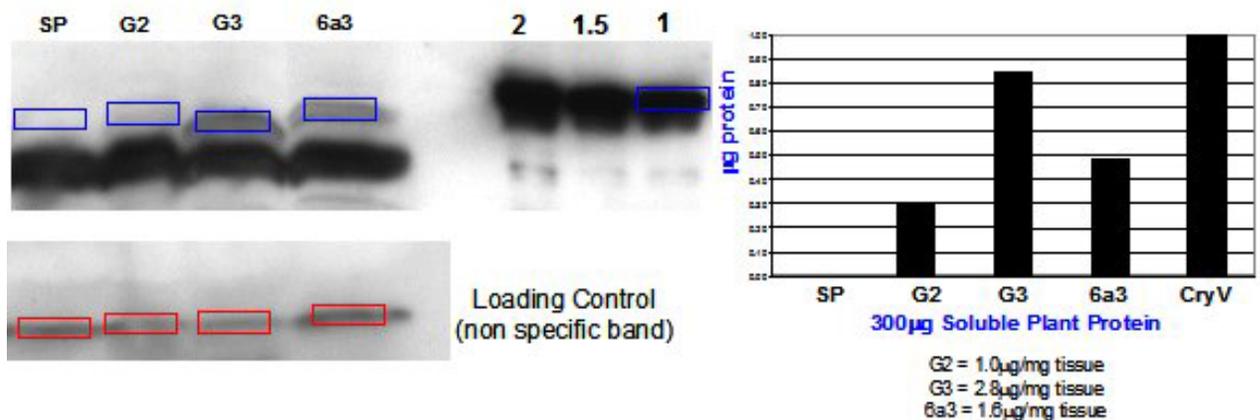


Figure 7. Western blot analysis to determine levels of cry1a1 protein in leaves of Spunta G2 and Spunta G3.

Discussion

The *Bt-cry1Ia1* from *Bacillus thuringiensis* used in our project, exhibits activity against both Lepidoptera and Coleoptera (Tailor et al. 1992), and was codon-modified to increase its expression level in plants. The codon-modified *Bacillus thuringiensis cry1Ia1* gene (obtained from Syngenta), encoding a delta-endotoxin protein, was introduced into the potato variety Spunta via *Agrobacterium tumefaciens*-mediated transformation (Li et al. 1999) and were shown to have high foliar *Bt* expression. In addition to the *cry1Ia1* gene, these potato lines also express the selectable marker gene, *npt2*, encoding the enzyme neomycin phosphotransferase from *E. coli*. Mohammed et al. (2000) identified these lines to have high tuber *Bt* expression under field and laboratory conditions.

Field trials were conducted in South Africa during the 2002-03 field season and results from the Ceres study show that the plants provide complete resistance to potato tuber moth under field conditions. Total tuber moth control was also found in the storage trials for eight months with Spunta G2 and G3. This results from our South African field trials are in agreement with results from our field and storage trials conducted in Egypt in 1999 and 2000. These studies show that Spunta G2 and G3 transformed potato lines are resistant to potato tuber moth and if they were available to growers they would easily fit into an integrated pest management program to manage potato tuber moth.

To allow growers to have access to Spunta G2 and G3 we must provide the required information that will allow for the commercialization of these lines. One part of the information required for commercialization is the molecular characteristics of the transformed lines. We have started the molecular characterization of the Spunta G2 and G3 lines have found that there is one copy of the gene in the G2 line and three copies in the G3 line and that the gene is intact in both lines. Protein expression from leaf tissue ranges from 1.0 to 2.8 µg/mg tissue in Spunta G2 and G3, respectively.

The potato group at MSU and its partners in Egypt and South Africa have developed and tested over 200 different potato lines and identify two lines, Spunta G2 and Spunta G3, which have the potential for commercial application. The benefits of this *Bt* potato to the farmer and end-users will be, reduced input costs (less insecticides purchased), increased marketable yield, improved quality, reduced post-harvest losses, reduced human exposure to pesticides, and less pesticide residues on potato tubers.

The potato group believes it is now time to go beyond the research stage and start the commercialization process for Spunta G2 and Spunta G3. This process will be divided into six components: Product Development, Regulatory File Development, Obtaining Freedom to Operate and Establishing Licensing Relationships, Marketing and Technology Delivery, Documentation of Socio-Economic Benefits, and Public Communication.

Project Highlights and Achievements

- ◆ Engineered vector constructs and expressed *Bt-cry1Ia1* gene in over 200 potato lines.
- ◆ Over 40 different *Bt*-lines were field tested in Michigan and/or Egypt and South Africa.
- ◆ Conducted studies to evaluate the effect of combining natural resistance mechanisms with *Bt-cry1Ia1*.
- ◆ First field trial of transgenic plants in Egypt in 1997. This field trial was the main incentive

for establishing biosafety guidelines for Egypt.

- ◆ First field trial of Bt-cry11a1 transformed potato plants was conducted in South Africa in 2001.
- ◆ Development of commercially acceptable Bt-Spunta lines to control potato tuber moth in the field and storage.
- ◆ Evaluated the efficacy of the *Bt-cry11a1* 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.
- ◆ **Intellectual Property Rights:**
 - Michigan State University (MSU) has used the Cry 11a1 gene from the Syngenta Company for the development of potato tuber moth resistant B.t. lines. MSU is in the process of negotiating two separate agreements/licenses from the Syngenta Company for the Cry 11a1 gene (formerly referred as Cry V gene).
 - The first license will be a research license to continue current research projects on B.t. potatoes in various countries and to share this technology for research purposes with other countries. This license will be between Syngenta and MSU, with MSU having rights to share this technology with other collaborators in developing countries for research purposes only.
 - The second license will be a royalty free license to commercialize this technology in South Africa and potentially other countries. This agreement will be between Syngenta and a partner country institution/company. MSU will review the terms of the license and play a role of facilitator to ensure that the license is fair and addresses stewardship issues. MSU will also assist in the development of regulatory files for the national government approvals. This license will be done on a country by country basis. We will start with South Africa as a first country to commercialize this technology. There are third party intellectual properties (IPs) involved in the B.t. potato project. These IPs include 35s promoter and NPT-II selectable marker gene. The Monsanto Company owns both of these IPs. The Syngenta Company will work with MSU and help facilitate in obtaining freedom to operate (FTO) agreement from Monsanto on these two pieces of technology.

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Presentations

- Santos-Gonzales, F., E. Grafius, W. Pett, and D. Douches. 2002. Control of potato tuber moth in field and storage in Egypt with Bt-transgenic potato lines. NC Branch Entomology Society of America. East Lansing MI.
- Two presentations to the National Needs Fellowship discussion group at MSU. The first titled “Transgenic Potatoes for Resource-poor Farmers in Developing Nations” in September of 2002. The second presentation “Commercialization of Bt-potatoes in Developing Nations” was given in March of 2003.
- Pett, W.L. “Biotechnology Applications in Agriculture: Bt-potato Case Study” presented at the

International IPM Short Course at MSU, June 2003.

Douches, D. and W. Pett. "Insect and Disease Resistance: Case study of Bt- Potatoes"
Presented at the International Agricultural Biotechnology Short Course at MSU, May 2003.

Travel of Project Personnel

Walter Pett traveled to South Africa in February of 2002 and visited with project partners at ARC-Roodeplaat.

Dave Douches visited the ARC-Roodeplaat Research Station, South Africa.

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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.

Development of a High Beta Carotene Variety of Mustard for Potential Development in a Food Based Approach to Reduce Vitamin A Deficiency in India— Final Technical Report (Monsanto)

Reporting period:

January 01, 2000 -- June 30, 2003.

Lead Principal Investigator

Cherian George, Ph.D., *Monsanto Co.*

Project Partners

Dr. Vibha Dhawan, TERI, India
Monsanto Research Centre, Bangalore
The Energy and Resources Institute (TERI)

Project Summary

In the late nineties, Monsanto developed canola varieties producing high amounts of beta-carotene through genetic engineering. It was subsequently proposed that this technology could be adapted to enhancing the beta-carotene content of Indian mustard varieties and the resulting high beta-carotene mustard varieties could be used to address vitamin A deficiency in India through a food-based approach. To this end, a research project was initiated in 2001 at Tata Energy Research Institute in India with financial support from USAID (through Michigan State University's ABSP I program) to explore the development of Indian mustard with elevated levels of Pro-vitamin A carotenoids. Preliminary results indicate that Indian mustard varieties can be genetically modified to express heterologous carotenoid biosynthetic genes and phenotypic demonstration of increased carotenoids content has been achieved. Additional work is needed to develop suitable high beta-carotene mustard varieties for potential deployment in a food-based approach to reduce vitamin A deficiency in India.

Overall Project Goal

The overall goal of this multi-phased project is to develop high beta-carotene mustard ('golden mustard') for potential deployment in a food-based approach to reduce vitamin A deficiency in India. The first phase of the project, which began in 2001 and ended in June 2003, was a feasibility study to determine whether the technology already developed for temperate varieties of canola could be transferred into Indian varieties of mustard.

Background

Vitamin A deficiency is endemic in the developing world, with the result that nearly 250 million people suffer chronic disease and even death due to lack of dietary access for this important micronutrient. Vitamin A malnutrition is prevalent in India. Most at risk are young children and women of childbearing age. Estimates are that 5-7% of Indian children are at various levels of Vitamin A malnutrition. Developing countries, often aided by donor programs, have attempted to address Vitamin A malnutrition through supplementation and fortification programs. While successful in some developing countries when these approaches have been adopted as a sustained effort, supplementation and fortification can be limited in their reach due to cost (especially of delivery) and by the narrow range of processed foods that are fortified, and additionally, may not reliably reach rural populations often dependent on very locally or self-produced crops and food. The new techniques of biotechnology offer an effective timely and scientifically feasible means to enhance the beta-carotene or Pro-vitamin A content of a wide range of common foods.

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

1. The transfer of technology developed for temperate varieties of canola into Indian varieties of mustard (*Brassica. Juncea*) and evaluate feasibility.
2. The training of Indian scientists in the various techniques and disciplines required in developing high beta-carotene mustard as a viable solution to vitamin A malnutrition in India.
3. The conduct of preliminary studies to evaluate the socio-economic use patterns associated with high beta-carotene mustard oil as a component of the Indian diet, especially in relation to vulnerable segments of society.
4. A preliminary assessment of intervention mechanisms which might be suitable for the introduction of beta-carotene enhanced mustard.
5. Organize workshops to increase awareness about the utility of a food-based, biotechnology approach for nutritional enhancement and to address concerns/issues among various stakeholders and also to understand the requirements of stakeholders and constraints if any for commercialization.

A follow-on program (phase II) would be initiated within India by the relevant scientific as well as development institutions, to develop economically viable high beta-carotene mustard germplasm and evaluate the use of this variety in a pilot, food-based, intervention scheme to address Vitamin A deficiency.

Project Partners

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

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

ABSP: This USAID-funded project based at Michigan State University was established in 1991 to facilitate the transfer of proprietary biotechnology practices and products to developing country institutions (both public and private) to address agricultural production constraints of local relevance. The program has also sponsored a significant biotechnology capacity building effort focused on, among other things, bio-safety and regulatory capacity building to establish legislative guidelines and protocols for product review in developing countries. ABSP activities and interventions have resulted in the establishment of bio-safety review frameworks in Egypt, Kenya and Indonesia. ABSP also has a demonstrated ability to programmatically and fiscally manage complex projects involving diverse partners.

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

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

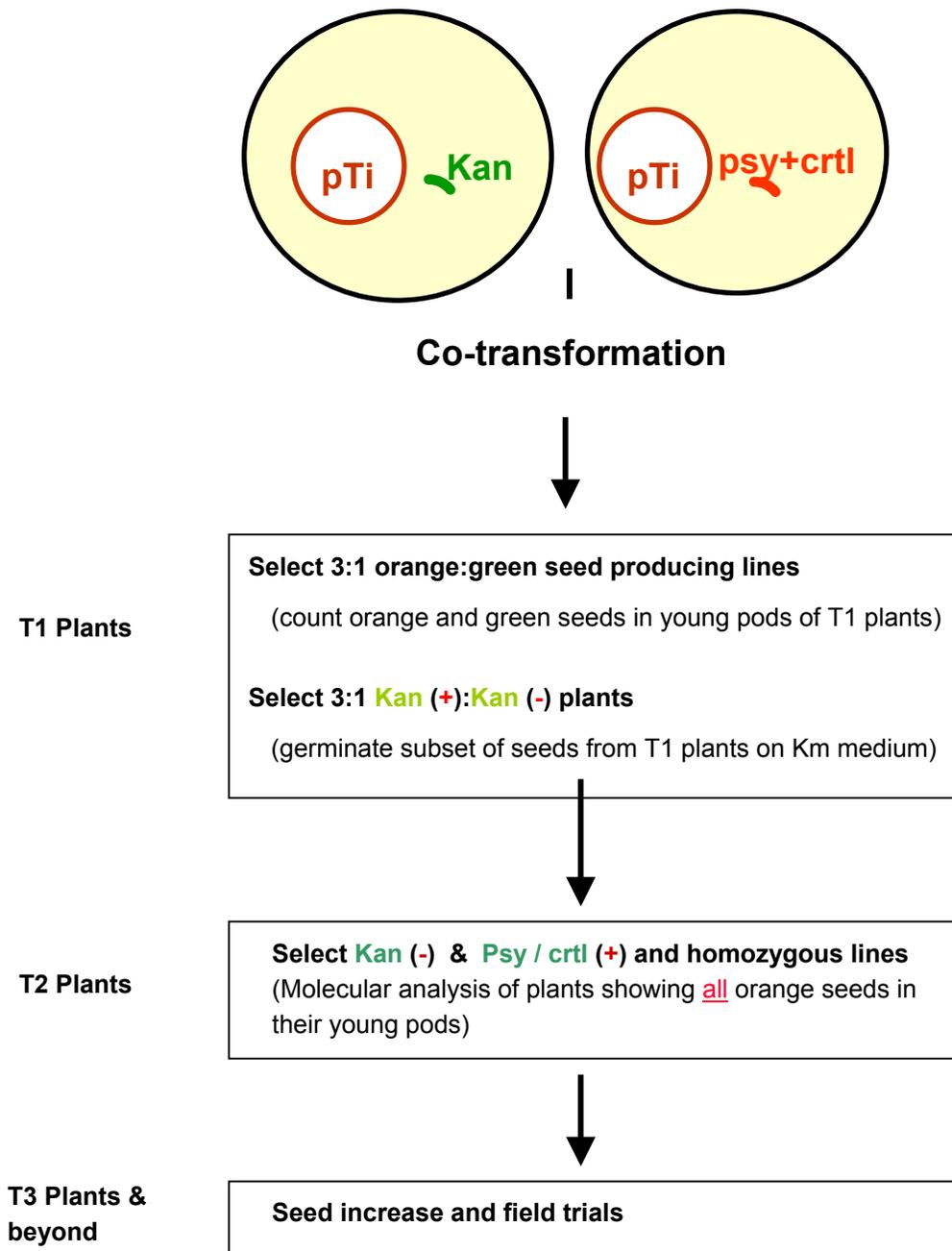
Research Progress

In plants, beta-carotene biosynthesis occurs in plastids. The enzyme that catalyzes the first committed step in carotenoid biosynthesis is phytoene synthase. Elevated expression of phytoene synthase in plastids of developing embryos of *Brassica napus* (canola) had been previously demonstrated to result in ca. 50-fold increase in the level of beta-carotene, in addition

to the increase in other nutritionally useful carotenoids in mature seeds. When oil is extracted from such seeds a large proportion of these carotenoids are partitioned into the oil fraction.

Mustard (*Brassica juncea*) is widely grown in India. There are several *Brassica juncea* mustard varieties grown in northern parts of India; among which three varieties, Varuna, Pusa Bold and RH30 are the most popular ones. These three genotypes (Varuna, Pusa Bold and RH30) were thus selected by TERI for genetic transformation, and TERI also supplied the necessary quantities of mustard seeds. Compared to canola (*Brassica napus*), the transformability of *Brassica juncea* is very poor. The genes coding for phytoene synthase (*Psy*) and phytoene desaturase (*CrtI*), both driven by a seed specific promoter, napin, were introduced into Indian mustard varieties to enhance the beta-carotene levels in their seeds. *Agrobacterium* mediated transformation was used to transfer the genes into mustard. In order to later remove the antibiotic selectable marker gene from the final product, a co-transformation technique (using two T-DNA) was used. In this approach, the kanamycin resistance selectable marker gene was placed in one of the binary vectors (Kan^R) and the genes of interest (*Psy* and *CrtI*) placed in a second binary vector (*Psy-CrtI*). Each vector was transferred into a separate *Agrobacterium* stock. *Brassica juncea* explants were co-transformed using the mixture of the above two agrobacterial stocks viz., one having the Kan^R binary and the other having the *Psy-CrtI* binary (Figure 1).

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



Genetic transformation and molecular screening of *Brassica juncea* was carried out at the Monsanto Research Center in Bangalore, India. Transgenic seeds (T2 generation) free of antibiotic marker gene and homozygous in psy/CrtI were shipped to TERI for greenhouse trials.

TERI obtained the necessary permits for transport of the transgenic mustard seeds to TERI research facilities and for the greenhouse trials from Institutional Bio-safety Committee (IBSC).

The seeds were grown at the National Phytotron Facility in Indian Agricultural Research Institute (IARI) as well as at the TERI greenhouse at Gual Pahari, Gurgaon and were taken to the T5 generation. Plans are being finalized at TERI to initiate a limited agronomic evaluation field trial of the T5 generation transgenic seeds in October 2003.

A recent decision by TERI to refocus their transformation efforts on these same leading mustard varieties and the expected organization of the effort to produce large numbers of independent events, would launch the project into the next phase of development.

Socio-economic Assessment

A socioeconomic assessment of mustard oil use and consumption among vitamin A deficient populations is essential for the development of intervention strategy and effective product introduction. The data on the prevalence of Vitamin A deficiency, food and oil consumption patterns in various Indian states was obtained from the reports of the National Nutrition Monitoring Bureau (NNMB) and compiled into a dossier. These first analyses lay the groundwork for the continued planning of effective intervention options.

Advisory Committee Meetings

An advisory committee of prominent Indian scientists, public/private sector partners was formed to guide the project. The committee met periodically at the TERI facility in New Delhi to set direction and monitor the progress of the project. Three meetings of the advisory committee were held during 2002-2003. Dr. S. Rao of the Department of Biotechnology, Government of India, facilitated the first two meetings. Dr. Vibha Dhawan of TERI facilitated the last meeting. Topics such as socio-economic assessment, nutritional impact, product introduction strategy, project management, greenhouse/field trials, etc. were extensively discussed at these meetings. Representatives from ABSP and USAID also participated.

International Symposium on “Biotechnology for Food & Nutritional Security”

An international symposium on “Biotechnology for Food & Nutritional Security” was organized by TERI on December 12-13, 2002 at New Delhi. The purposes of the symposium were:

1. To foster dialogue between researchers and policy makers working in the areas of biotechnology, food and nutrition.
2. To enhance the communication among various stakeholders involved in the high beta-carotene mustard project.
3. To increase the public and professional awareness of the benefits of plant biotechnology using high beta-carotene mustard and other nutritionally enhanced crops as examples.
4. To help define the path forward for the high beta-carotene mustard project.

The symposium was a success and it met all the above objectives.

Other Activities in 2002-2003

1. Dr Vibha Dhawan (from TERI) gave a presentation on Food Security and Basic Human Needs at the Delhi Sustainable Development Summit 2002 at TERI, New Delhi, India.
2. Dr Malathi Lakshmikumaran (from TERI) gave a presentation on Improvement of nutritional quality traits in crop plants on May 25, 2002 at the one-day workshop on "Nutritional Security in the 21st Century" in the memory of great agricultural scientist Professor B. P. Pal at TERI, New Delhi, India.
3. At BIO 2002, An International Biotechnology Convention and Exhibition, Toronto, Canada, June 10, 2002, Dr Dhawan gave a presentation entitled "Biotechnology for developing Countries: An important Tool in Sustainable Development".
4. Dr Dhawan gave a presentation on "Identifying Products of Modern Biotechnology: India" at "The Next Harvest, Advancing Biotechnology's Public Good: Technology Assessment, Regulation and Dissemination", Organized by ISNAR at The Hague, The Netherlands, October 7-9, 2002.
5. Dr Dhawan delivered a lecture on "Public-Private Sector Partnership and IP Issues" in a Conference entitled "More People, Less Land – Technology's Role in Sustainable Agriculture in Asia" organized by Croplife Asia at Bangkok, Thailand on November 5, 2002.
6. Dr Dhawan gave a talk on "Biotechnology: The perspectives from developing countries and partners: towards a global strategy in plant biotechnology for food security and poverty alleviation" at Indianapolis, Indiana, USA sponsored by FAO-ABSP-MSU. November 12-14, 2002
7. Dr Cherian George (from Monsanto) gave a presentation on nutritional enhancement of crops through biotechnology at the international symposium on "Biotechnology for Food & Nutritional Security" on December 12-13, 2002 at New Delhi, India.
8. A meeting of eminent nutritionists from India and abroad was organized on February 26, 2003. In the meeting, the strategies to assess the nutritional aspects of the genetically modified mustard oil, development of delivery vehicle for the oil and creating public awareness were discussed.
9. Dr Vibha Dhawan made a presentation on "Golden Mustard: Technique to Technology" at the "Workshop on Priority Setting for ABSP II Projects in India" organized by Cornell University in Hyderabad, India on April 9, 2003.
10. Dr Dhawan attended the Workshop on "Models of Food Safety Assessment of Transgenic Crops" on May 6-8, 2003 at Washington DC.
11. Dr Vibha Dhawan presented the mustard project at the Final meeting of the ABSP external Board of Directors at Washington on May 9, 2003.
12. Dr. Cherian George presented the "Safety Assessment Strategy for Golden Mustard" at the workshop on "Models of Food Safety Assessment of Transgenic Crops" on May 6-8, 2003 at Washington DC.
13. Dr Vibha Dhawan gave a presentation entitled "Golden Mustard: Development of high beta carotene mustard oil" at Indo-US Agricultural Biotechnology Conference on Nutritional Enhancement & Abiotic Stress Tolerance on May 15, 2003, New Delhi

Development of a High Beta Carotene Variety of Mustard for Potential Development in a Food Based Approach to Reduce Vitamin A Deficiency in India— Final Technical Report (TERI)

Reporting period:

January 01, 2000 -- June 30, 2003.

Lead Principal Investigator

Dr. Vibha Dhawan, TERI, India

Project Partners

Monsanto Research Centre, Bangalore

The Energy and Resources Institute (TERI)

Overall project goal

The overall goal of the project is to develop a suitable variety of locally adapted Indian mustard (*Brassica juncea*) with enhanced beta-carotene content and to initiate the use of this variety in a pilot, food-based, intervention scheme to address Vitamin A deficiency and, accordingly child mortality.

Keeping in view the complex nature of the project, its execution was planned in two phases:

Phase I: To June 2003

Phase II: July 2003 – June 2008

As the first phase of this project ended on June 2003, this report summarizes the progress made during the course of the project.

Importance of the problem/justification for project

The successful completion of this study would result in development of a *Brassica juncea* variety with high beta-carotene content. This will go a long way in addressing the problem of Vitamin A deficiency among children in India. Subsequently, this approach could be replicated for other crops and locales to address Vitamin A deficiency and childhood mortality, at large, in the developing world.

Project background

Micronutrient deficiencies are widespread, affecting more than one-third of the world's population. Vitamin A, iron, iodine and folate are some of the micronutrients that are deficient usually. Lack of

this vitamin causes many disorders ranging from minor skin infections, night blindness, permanent blindness, increased morbidity and mortality, impaired immune response anemia, increased pulmonary infection, diarrhea and higher infant mortality.

Vitamin A is directly available from foods of animal origin, such as breast milk, liver, eggs, butter and cow's whole milk. Its precursor, beta carotene is present in plant foods such as green-leafy vegetables, orange and yellow fruits such as carrot, spinach, tomatoes, chillies, papaya, melon and mango, which gets converted into vitamin A in the body.

The carotene levels of green leafy vegetables are very low and their bioavailability is even lower. Furthermore, in drought prone regions of the world, availability of fruits and vegetables is highly seasonal. Adverse ecological conditions, cultural practices including food habits and food taboos, combined with general poverty, low availability and low control of resources contribute to inadequate intake of Vitamin A rich or carotene-containing foods. This leads to Vitamin A deficiency particularly in infants, children, expecting and lactating mothers, in India and other developing countries. Worldwide, nearly 250 million people including over 100 million pre-school age children suffer from chronic diseases and death, which may be attributed to Vitamin A deficiency. According to rough estimates, 5-7% children in India suffer from vitamin A deficiency and 30,000-40,000 children below five years of age face the risk of preventable blindness due to this deficiency. Many people have sub-clinical levels of vitamin A deficiency and may not be aware about it. Quality of life among affected children is also severely compromised as a result of abnormalities of the eye, which result in varying degree of blindness. Half of the blindness in children around the world can be attributed to vitamin A deficiency. Vitamin A deficiency is also widespread among reproductive-aged women. The global toll of maternal death is nearly 600,000 each year, with the vast majority of these women localized in the developing countries and dying from largely preventable causes. For example, Vitamin A that was provided to vitamin A deficient pregnant women under supplementation programs in Nepal reportedly reduced pregnancy-related deaths by an average of 44%. Vitamin A, stored normally in the liver, is crucial for an effective and functional immune system, protecting the integrity of epithelial cells lining the skin, the surface of the eyes, the inside of the mouth and the alimentary and respiratory tracts. When the effectiveness of the immune system defense breaks down, as in a Vitamin-A deficient child, susceptibility to infection increases.

Rationale for approach

The intake of vitamin A in the diet of the target group of people can be increased by supplementation, fortification or development of carotene rich foods. For example, a National Vitamin A prophylaxis program (a supplementation program) has been operational in India for over 25 years. However, this approach has not been very successful due to poor implementation and monitoring. Supplementation in the form of pills or capsules has led to more problems like over-dosage. Excess intake of vitamin A causes vomiting, anorexia, sleeping disorders, and enlarged liver and is toxic to pregnant women as it can harm the fetus. For example, Vitamin A supplementation of the type routinely provided to young children in developing countries (200,000 IU at 4-6 month intervals) could be a possible risk to a developing fetus. Other programs in countries have used Vitamin A fortification of common foods, such as sugar in Ghana and milk and flour in India, as an alternative to direct supplementation. The results of supplementation/fortification programs may be variable, with failures generally resulting from the

inability of resource poor governments to sustain programs economically and organizationally over a period of time in the absence of donor funding. Also, the fortified foods available in the market come with a price tag that is unaffordable by many sections of the society. Therefore, bioavailability data and socio-economic use patterns support a food-based approach to develop carotene-rich foods as one of the viable alternatives to address worldwide Vitamin A deficiency.

Since conversion of carotene to vitamin A occurs in the body as needed, safety issues surrounding dose regimens for “at risk” members of a population are minimized. Also, development of beta-carotene enriched crops, such as mustard, offer a reliable, economical, culturally compatible and locally available source of beta-carotene which may be integrated into even the most rudimentary existing crop production schemes to ensure access for the poorest members of the society.

Previous research

As opposed to the recent past, the new techniques in biotechnology offer an effective, timely and scientifically feasible means to enhance the carotenoid contents of common foods in a targeted and sustainable approach and the “proof-of-concept” for this proposal is currently being demonstrated by Monsanto with North American varieties of canola. The choice of mustard for India is a logical one, given that the Indian government has expressly stated its preference for a food-based delivery system and has initiated an approach in red palm oil which would be targeted to populations in the southern part of the country where red palm oil is the oil of choice. This present study for mustard oil would complement the Indian government’s ongoing programs but would focus only on the northern parts, where mustard oil is preferred. In addition, pending positive results for agronomic performance, it is likely that farmers and families, in the wake of an effective education campaign, would preferentially adopt this new variety as a value-added yet economical source of nutrition which could easily be substituted as a source for the existing consumption of mustard oil.

Using recombinant DNA technology, Monsanto has produced a variety of canola, which displays a significantly enriched carotenoid composition in the oil (HCCO). On the other hand, TERI has developed or has access to a number of commonly cultivated varieties of Indian mustard. Therefore, it was decided to combine the technical expertise available at the two research institutes to develop beta-carotene enhanced mustard varieties. Under this project Monsanto has licensed, royalty-free, use of its carotenoid technology for the development of high beta-carotene mustard. The TERI, with the assistance of ABSP, will develop mustard varieties expressing high levels of beta-carotene to reduce vitamin A deficiency in India.

Objectives

1. The transfer of technology developed for temperate varieties of canola into Indian varieties of mustard (*Brassica juncea*)
2. The subsequent varietal testing under Indian laboratory and field conditions to assess agronomic performance and nutritional value
3. The training of Indian scientists in the various techniques and disciplines required in developing high beta-carotene mustard as a viable solution to vitamin A malnutrition in India
4. A preliminary assessment of intervention mechanisms that might be suitable for the introduction of beta-carotene enhanced mustard

5. Organize workshops to increase awareness about the utility of food-based, biotechnology approach for nutritional enhancement and to address concerns/issues among various stakeholders and also to understand the requirements of stakeholders and constraints if any for commercialization.

Research Progress

Brassica juncea is widely grown in India. There are several *B. juncea* varieties grown in northern parts of India; among which three varieties, Varuna, Pusa Bold and RH 30 are the most popular ones. Two of these varieties were thus selected by TERI for transformation, who also supplied the necessary quantities of mustard seeds to Monsanto. Compared to canola (*B. napus*), the transformability of *B. juncea* is very poor. The genes coding for phytoene synthase (*Psy*) and phytoene desaturase (*Crt1*), both driven by a seed specific promoter, napin, were introduced into Indian mustard varieties to enhance the beta-carotene levels in their seeds. *Agrobacterium*-mediated transformation was used to transfer the genes into mustard. In order to remove the antibiotic selectable marker gene later from the final product, a co-transformation technique (using two T-DNAs) was used. In this approach, the kanamycin resistance selectable marker gene was placed in one of the binary vectors (Kan^R) and the genes of interest (*Psy* and *Crt1*) placed in a second binary vector (*Psy-Crt1*). Each vector was transferred into a separate *Agrobacterium* stock. *B. juncea* explants were co-transformed using the mixture of the above two agrobacterial stocks viz., one having the Kan^R binary and the other having the *Psy-Crt1* binary. When oil is extracted from such seeds large proportions of these carotenoids are partitioned into the oil fraction. The *psy* gene serves as a phenotypic marker for selection as the immature seeds expressing the engineered *Psy* turn orange in color, due to the accumulation of β -carotene, as opposed to normal green seeds. Hence, T1 plants having a single copy insert of *Psy* could be easily identified on the basis of siliques containing orange and green seeds in the ratio of 3:1. Seeds were harvested from only those T1 plants that show 3:1 ratio to raise the next generation, i.e. T2. In the T2 generation, the plants are analyzed by PCR and Southern hybridization and those plants that have *psy* + *crt1* genes but free from *nptII* are used to raise the next generation. Null segregants are essential for comparison of traits.

Out of a total of over 100,000 explants from the three varieties that were infected during 2000-01 at Monsanto Research Centre at Bangalore, fifty viable shoots were recovered. The fifty shoots yielded 8 orange seed producing lines (seeds turn orange because of the higher carotenoid content). About 100 T2 generation plants from each of the eight orange seed producing lines were screened for the absence of antibiotic marker gene. Approximately 25 T2 plants were found to be free of antibiotic marker gene and homozygous in *Psy* and *Crt1*.

After completing the laboratory studies related to *Agrobacterium*-mediated transformation of two Indian varieties of *Brassica juncea* i.e., Pusa Bold and Varuna and raising the transformants up to the T2 stage, 50 transgenic seeds and 50 null segregants (T3 generation) of each of the five *Brassica juncea* lines as described below were transferred by Monsanto to TERI for further research:

- i. PB 12010-2- *Psy* + *Crt I* & Null segregants
- ii. PB 12010-8- *Psy* + *Crt I* & Null segregants
- iii. V 12010-2- *Psy* + *Crt I* & Null segregants
- iv. V 12010-3- *Psy* + *Crt I* & Null segregants

v. V 12010-4- Psy + Crt I & Null segregants

1. Analysis of Transgenic seeds of Varuna and Pusa Bold for agronomic traits (homozygosity of the gene of interest and its stable inheritance)

Complying with the guidelines of Department of Biotechnology, Govt. of India, 10 seeds each of the transgenic lines and their null segregants were deposited with Head, Germplasm Conservation Division, NBPGR, New Delhi. The remaining T3 seeds were split into two lots of 20 seeds each and grown simultaneously at two places: 1) at the National Phytotron Facility in IARI, New Delhi and 2) inside the greenhouse at TERI's Gual Pahari campus in Gurgaon to raise the next generation of plants (T3). Prior permission was obtained from the IBSC at IARI and TERI for growing the plants at the two locations.

The plants were observed periodically to record any variation from the control. There was variation in the frequency of germination of the various lines and also the intensity of orange color in the cotyledons. When the plants started flowering, they were bagged to obtain selfed seeds. Finally, the selfed and open-pollinated seeds (T4) were harvested from the plants and the seed yield was recorded for various lines growing at the National Phytotron Facility (Table 1) and inside the greenhouse at TERI (Table 2). There was a variation in the seed yield of plants growing at National Phytotron Facility and the greenhouse at TERI as evident from the data given in Tables 1 and 2. While some transgenic lines such as V 12010-2 and V 12010-4 exhibited seed sterility at the National Phytotron Facility, all the lines grown in TERI's greenhouse were fertile. However, the seed yield per plant was more at National Phytotron Facility than that in TERI.

Selfed seeds harvested from the T3 generation were sown again for bulking up for one more cycle. (T4 generation) Permission for growing the T4 generation at National Phytotron Facility was accorded by the IBSC of IARI in its meeting held on March 21, 2003. The T4 seeds were sown at the National Phytotron Facility in IARI, New Delhi on April 24, 2003 to raise plantlets. In this generation too, some lines such as V12010-4 exhibited poor germination. For T4 generation, a larger number of plants for each line would be grown so that we could obtain maximum possible seeds. The first limited open field trial will be carried out at TERI in October 2003 using T5 seeds.

2. Genetic Transformation and the analysis of putative transgenic lines for stable transformants through PCR and segregation of color

The methodology that was followed for the development of transgenic Pusa Bold and Varuna was also applied for YSRL. However, there seem to be some problems with the genetic transformation of YSRL. The shoots obtained on selection medium (containing antibiotics) appear bleached, suggesting that these were not transformed. Presently, transformation is being carried out with Pusa Bold and Varuna to generate additional transformed lines.

Table 1. Seed yield from T3 lines grown at National Phytotron Facility*Date of sowing: October 7, 2002; Date of harvest: February 5, 2003*

Line	Total no. of seeds	No. seeds germinated	Plants forming seeds	Range of no. of seeds per line	Mean no. of seeds*	Mean Seed weight (g)
PB control	15	14	14		600	2.93
PB12010-2	20	7	5	200-400	300	1.52
PB12010-2 (null)	4	4	4		150	1.64
PB 12010-8	20	6	4	50-300	200	1.14
PB12010-8 (null)	4	2	2		150	1.89
Varuna control	11	8	8		400	3.18
V-12010-2	20	13	0		0	0
V-12010-3	20	9	1		400	4.85
V-12010-4	20	8	2	200-400	300	2.74

Table 2. Seed yield from T3 lines grown at greenhouse at TERI*Date of sowing: October 19, 2002; Date of harvest: April 26, 2003*

Line	Total no. of seeds	No. of seeds germinated	Plants forming seeds	Range of number of seeds per line	Mean Number of seeds*	Mean Seed weight (g)
PB control	6	6	6		130	2.05
PB12010-2	20	8	8	60-200	75	1.0
PB12010-2 (null)	20	20	20		49	0.60
PB 12010-8	20	12	12	30-150	70	0.49
PB12010-8 (null)	20	19	19		55	0.71
Varuna control	9	7	7		125	1.75
V-12010-2	30	21	21	9-100	50	0.59
V-12010-2 (null)	20	17	17		55	0.59
V-12010-3	25	19	19	20-100	65	0.60
V-12010-3 (null)	20	14	14		85	0.75
V-12010-4	30	15	15	20-200	50	0.63
V-12010-4 (null)	30	19	19		45	0.55

* Mean number of seeds denotes the mean of the total number of seeds collected from all the plants of one transformed line.

3. Socio-economic assessment

Social-economic analysis of mustard oil use and consumption pattern among affected households and communities is necessary to develop an intervention strategy for effective product introduction.

During the course of the study the data about the prevalence of vitamin A deficiency, food and oil consumption patterns in various states was obtained from the reports of National Nutrition Monitoring Bureau (NNMB) and compiled. The reports from NNMB cover the states of Kerala, Tamilnadu, Karnataka, Andhra Pradesh, Maharashtra, Gujarat, Madhya Pradesh, Orissa and West Bengal only. However, most of these states (except for West Bengal and Orissa) represent populations that do not consume mustard oil. However, they do use mustard seeds in good amount as condiments.

Discussion / Implications

Though it would be premature to draw any conclusion at this stage, it seems that the transgenic lines had a lower seed yield as compared to the controls. However, one would be able to draw more concrete conclusions only after the open field trial.

In achieving transformation of *B. juncea* cv. YSRL there seem to be two bottlenecks. a) The Agrobacterium strain is slow growing and less virulent. b) YSRL is less amenable to transformation.

Moreover, one has to see that YSRL (A low erucic acid variety) is not popular among farmers as yet and may not be the best for the targetted consumers in this project. It may also add a price tag on the product. Therefore, we feel that we should concentrate on popular varieties of mustard (Pusa Bold and Varuna).

Highlights of significant achievements & Future Plans

Genetic transformation would be concentrated on varieties like Pusa Bold and Varuna.

Open field trial of transgenic seeds

- ◆ The T5 seeds would be used to conduct a limited trial for bulking of seeds as well as to collect agronomic data and conduct ecological and environmental studies.

Food safety studies

- ◆ Nutritional (carotenoid content)
- ◆ Besides carotenoid and fatty acid profiles, levels of other compounds such as tocols and tocopherols would be estimated.
- ◆ In addition studies pertaining to bioavailability of beta-carotene will be performed.

The nutritional studies would be performed in collaboration with Dr Shiv Kumar, National Institute of Nutrition, Hyderabad.

- ◆ Toxicity tests

Toxicological studies and allergenicity tests would be carried out at Industrial Toxicology Research Centre, Lucknow along with other institutes of repute in India.

- ◆ Stability tests

Studies regarding the stability of beta-carotene in the oil with respect to various cooking methods employed traditionally in India will be carried out.

Socio-economic assessment

- ◆ This would be done with the help of field surveys, questionnaires, etc. Effective delivery mode for the oil and promotional strategies will be designed for higher public acceptance.

Public outreach and communications

- ◆ While the work towards development of transgenic mustard is still in progress, discussions to create awareness among government, industry, farmers and NGOs have already been initiated. Mid-term workshops to discuss benefits, issues and concerns related to nutritional modification of crops via agriculture biotechnology are also being organized. We propose to continue with the holding of such workshops and discussions in future as well.

- ◆ The penetration and growth of a new project depends entirely on its acceptance among the consumers. Since GM crops are new to India with only a single transgenic crop (Bt Cotton) been released commercially that too only recently, there are still many apprehensions, concerns, doubts and fears with respect to biosafety and ecological risks of GM crops. The problem has been further compounded due to lack of awareness on the subject among the masses. To deal with this situation it would be important to apprise people about the facts which, are based on scientific knowledge rather than the myths. This will provide better understanding about GM crops to the consumers thereby ensuring greater acceptability of the product.

Development of technology delivery mechanisms

- ◆ Much of the GM crops that have being developed and released so far have benefited the producers directly much more than the consumers. For example, transgenic crops those are resistant to insects, diseases, herbicides, stress, etc. In contrast “Golden mustard” is one crop which would be of direct consequence to the consumers whose number of beneficiaries would exceed that of the producers by several fold. Consequently, the number of stakeholders in the given situation would be much higher and accordingly the strategy that would have to be adopted for the release of this crop will have to be very different. Also, a much aggressive awareness campaign will have to be launched to educate consumers about the benefit of the product.

Government approvals at different stages of product development

- ◆ In accordance to the norms of Department of Biotechnology, Govt. of India, time to time approval from IBSC, RCGM and GEAC is imperative. In addition, approval will also be sought from the USAID Biosafety Committee before the release of the product. All formalities required to get a transgenic variety released would be completed.

IPR Issues

- ◆ The major issues in this context are transformation technology, genes, promoters, plasmid constructs, PCR techniques, PVP/extant varieties and statistical methods. It has to be seen that no IPRs are violated. An IPR audit committee would be formulated for this. The PVP Act will have to be taken into consideration for the Indian scenario.

Commercial release

- ◆ Tie-up with the industry would be absolutely essential for the commercialization of the product. For this, TERI will enter into a dialogue with various seed companies for bulking-up and distribution of transgenic seeds among the farmers. Simultaneous efforts would be made to involve companies engaged in edible oil business for a buy-back arrangement with farmers.

Publications during the reporting period

The Proceedings of the International Symposium on Biotechnology for food and nutritional security is under preparation and would be published by October 2003.

Travel of project personnel and visits by project partners

- ◆ At BIO 2002, An International Biotechnology Convention and Exhibition, Toronto, Canada, June 10, 2002, Dr Dhawan gave a presentation entitled "Biotechnology for developing Countries: An important Tool in Sustainable Development".

- ◆ Dr Dhawan gave a presentation on "Identifying Products of Modern Biotechnology: India" at "The Next Harvest, Advancing Biotechnology's Public Good: Technology Assessment, Regulation and Dissemination", Organized by ISNAR at The Hague, The Netherlands, October 7-9, 2002.
- ◆ Dr Dhawan delivered a lecture on "Public-Private Sector Partnership and IP Issues" in a Conference entitled "More People, Less Land – Technology's Role in Sustainable Agriculture in Asia" organized by CropLife Asia at Bangkok, Thailand on November 5, 2002.
- ◆ Dr Dhawan gave a talk on "Biotechnology: The perspectives from developing countries and partners: towards a global strategy in plant biotechnology for food security and poverty alleviation" at Indianapolis, Indiana, USA sponsored by FAO-ABSP-MSU. November 12-14, 2002.
- ◆ An International Symposium on "Biotechnology for Food & Nutritional Security" was organized by TERI on December 12-13, 2002 which focused on various aspects pertaining to the commercial release of golden mustard.
 - i. This symposium was held with the following objectives:
 - ii. To bring together the various professionals (researchers & policy makers) working in fields related to biotechnology, food security and nutritional security.
 - iii. To hold meaningful dialogues between various stakeholders involved in this project.
 - iv. To create awareness in the media and the masses about the rationale behind the project and the benefits of this technology to humankind.
 - v. To help us define our path for implementation of the project as it involves complex issues related to biosafety, environment, socio-economic implications, regulatory studies, role of various stakeholders from the government to the consumer, and finally public awareness and acceptance.

This symposium served as an ideal platform for eminent people from various fields to have a holistic view of this philanthropic project and provide solutions, thus helping us to define our path in a more concrete manner. It was also attended by scientists from USAID and Monsanto.

- ◆ A meeting of eminent nutritionists from India and abroad was organized on February 26, 2003. In the meeting, the strategies to assess the nutritional aspects of the genetically modified mustard oil, development of delivery vehicle for the oil and creating public awareness were discussed.
- ◆ Dr Dhawan made a presentation on "Golden Mustard: Technique to Technology" at the "Workshop on Priority Setting for ABSP II Projects in India" organized by Cornell University in Hyderabad on April 9, 2003.
- ◆ Dr Dhawan attended the Workshop on "Models of Food Safety Assessment of Transgenic Crops" on May 6-8, 2003 at Washington, USA.
- ◆ Dr Dhawan presented the mustard project at the Final meeting of the ABSP external Board of Directors at Washington on May 9, 2003.
- ◆ Dr Dhawan gave a presentation entitled "Golden Mustard: Development of high betacarotene mustard oil" at Indo-US Agricultural Biotechnology Conference on Nutritional Enhancement & Abiotic Stress Tolerance on May 15, 2003, New Delhi.

In addition, three meetings of the Advisory Board of the Project have been held on March 7, 2002, December 14, 2002 and June 3, 2003, respectively. These meetings were attended by the

project partners such as Monsanto, USAID and MSU as well as other eminent scientists who are members of the advisory Board.

Development of virus resistant cucurbits—Final Technical Report (MSU)

Reporting period: 1995 - 2003

Principle Investigator

Dr. Rebecca Grumet, MSU

Project partners:

Dr. Atek Sadik, AGERI, Egypt

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

Dr. Asih Kartasih Karjadi, Center for Horticultural Research, Indonesia

Project Goal

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, our primary collaborating country, 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 mosaic 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).

Previous Research

Research Objectives

- I. Produce melon genotypes that express the ZYMV-CP gene; characterize the nature and extent of resistance and perform field trials.
- II. Develop cucurbit transformation systems.
- III. Perform stress testing of transgenic cucumber lines.
- IV. Risk assessment studies.

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 more 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) dehydration 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	χ^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. Stress 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.

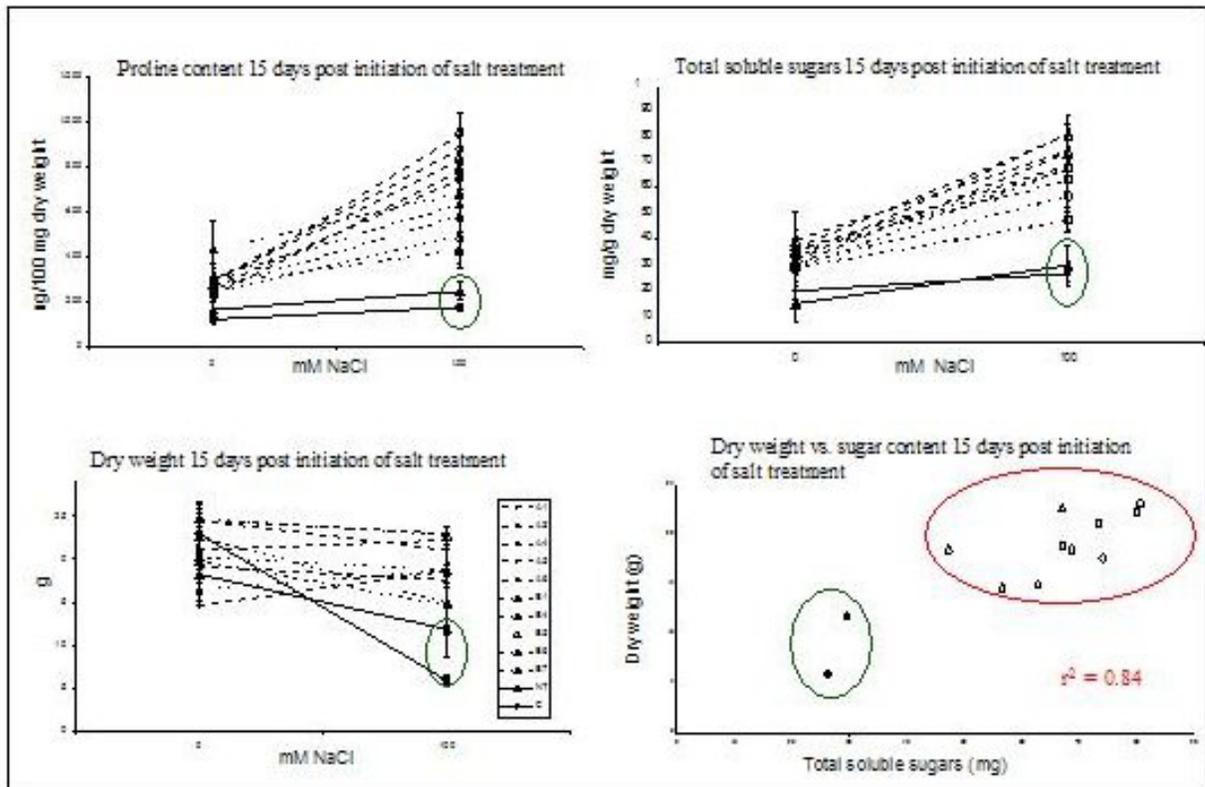


Figure 4. Response of transgenic cucumbers transformed with CBF1 or CBF3 genes to treatment with 100 mM NaCl. Each point is the mean of six replicate plants + S.E.

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 significant 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 movement 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".

Research Progress 10/2002 - 6/2003.

III. Stress testing of transgenic cucumber lines.

The primary effort in the past several months has been stress testing of the transgenic CBF lines and preliminary characterization of the *M6PR* lines. Salt stress trials in the greenhouse were repeated a third time with the *CBF* lines with analogous results to those described earlier, verifying reproducibility of the observed physiological responses (proline, sugars) and importantly, protection against salinity stress as measured by fresh weight and dry weight accumulation. These results have been submitted for presentation at the national meeting of the American Society for Horticultural Science (Tawfik and Grumet, 2003).

The next step is to test the *CBF* lines under field conditions. A field trial is currently in progress at the MSU Horticulture Teaching and Research Station (Figure 5). All appropriate university, state and federal approvals were obtained. The plants were planted into bags of sand to minimize damage to the field and facilitate salt stress conditions as determined from greenhouse experiments. Importantly, field trials will allow for testing effect on fruit production. Salt stress treatments are underway and the first field trial is beginning to set fruit. We are preparing the plant materials to perform a second replication of the field experiment in the same location (if frost does not occur too early).



Figure 5. Salt stress experiment testing transgenic *CBF*-cucumbers in the field, 2003.

Preliminary experiments also were performed to determine appropriate conditions for drought stress experiments in the greenhouse. Variables tested included pot size and type, soil mix, and various water stress treatments. Based on those results, an experiment is currently in progress using three *CBF* genotypes planted into ca. 12 liter pots filled with 1:1 Baacto soil mix: sand. Plants will be subjected to a three cycles of withholding water until the point at which control plants wilt, followed by re-watering.



Figure 6. Drought stress trial of *CBF* transgenic cucumbers in the greenhouse.

Training & policy-related activities

R. Grumet has participated as a lecturer and panel discussant in the Biosafety Workshops being organized by Dr. Karim Maredia.

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Travel

- 1998: Rebecca Grumet traveled to Egypt for planning workshop.
- 1997: Rebecca Grumet as part of group to Indonesia to explore research collaborations
- 1996: Dr. Asih Kartasih Karjadi, Center for Horticultural Research, Indonesia
One month at MSU with R. Grumet to learn *Cucumis* tissue culture and transformation

Equipment

The only non-expendable equipment purchased with ABSP funding was a computer approximately five years ago. That computer is still in use in the laboratory.

Development of Virus and Disease Resistant Cucurbits—Final Technical Report (Cornell University)

Reporting period: 1995 - 2003

Principal Investigator

Dr. Molly Jahn, Dept. of Plant Breeding, Cornell University

Other Principal Investigators

Henry M. Munger and R. Provvidenti, Cornell University

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 adapted 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 attendance 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 resistance 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. We currently have licenses and/or MTAs in place for varieties developed with ABSP support with seed companies or public sector variety development programs in Egypt, South Africa, Brasil, Philippines, Indonesia, India, and Pakistan.

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 and support the international collaborations and travel necessary to deliver varieties to the developing world.

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Technical Support and Backstopping to the Association for Strengthening Agricultural Research in East and Central Africa (ASARECA) Biotechnology and Biosafety Initiative—Final Report

Reporting Period

March 15, 2002 – December 31, 2002

Lead Principal Investigator

Dr. Joel I. Cohen, International Service for National Agricultural Research (ISNAR)

Scope of Work

This report summarizes achievements under Subagreement No. 612924 between Michigan State University (MSU) and ISNAR, with financial support provided by the U.S. Agency for International Development (USAID) Prime Grant No. DAN-A-00-91-00126-00 to MSU. The report covers the period March 15, 2002 to December 31, 2002.

In order to expand its expertise and resources, the USAID-supported Agricultural Biotechnology Support Project (ABSP) engaged the services of Dr. Joel Cohen, Principal Research Fellow and Manager, ISNAR Biotechnology Service, International Service for National Agricultural Research (ISNAR), as a technical consultant to ABSP, in particular to assist with technical backstopping to ASARECA's biotechnology initiative. Dr. Cohen was requested to work directly with ASARECA's biotechnology coordinator and closely with the ABSP Management Team, in planning the various activities, attending Working Group meetings and workshops as well as assisting with the compilation of the final program proposal from ASARECA.

In addition to Dr. Cohen's services, Mr. John Komen and Dr. José Falck Zepeda from ISNAR were made available for drafting reports and proposals, contributing to meetings, and ensuring continuity of the project. For further expertise in biosafety and regulation, Dr. Donald Mackenzie of AGBIOS was also supported. Thus, ISNAR ensured a wide spectrum of expertise, to match the particular needs of the various working and stakeholder group meetings for ASARECA, as well as to provide continuous support to AID in this process.

The Scope of Work, as included in the Subagreement between MSU and ISNAR, for this activity included:

- 1. ASARECA Biotechnology and Biosafety Working Group Meeting, April 2002**
 - Develop WG Agenda
 - Determine ASARECA approach towards a biotechnology and biosafety initiative
 - Develop criteria to determine priorities
 - Presentation and analysis of biotechnology review paper
 - Plan stakeholders workshop for May 2002

- 2. Biotechnology Stakeholders Workshop, June 2002**
 - Broaden discussion on completed consultant reports
 - Agree on priorities, mission and objectives
 - Prepare strategic plan and recommendations to ASARECA Committee of Directors

3. **ASARECA Working Group Meeting on Biosafety, June 2002**
 - Discuss consultant paper on biosafety, analyze and prioritize
 - Recommendations to ASARECA CD
 - Plan Biosafety Workshop for August 2002
 4. **Biosafety Workshop, August 2002**
 - Agree on priorities
 - Develop vision, mission and objectives
 - Prepare strategic plan and disseminate to stakeholders for comments
 5. **Meetings with ASARECA Coordinator for Biotechnology and Biosafety, August-September 2002**
 - Develop draft proposal for ASARECA Initiative on Biotechnology and Biosafety
 - Request comments by USAID officers
 6. **ASARECA Working Group Meeting, September 2002**
 - Discuss draft ASARECA proposal
 - Incorporate comments and changes following review by USAID
 - Review second draft
 - Compile final proposal
7. **Submit final proposal**

Summary of Activities and Achievements

1. ASARECA Biotechnology and Biosafety Working Group Meeting, April 2002

The meeting was held from April 15-17 2002, in Nairobi, Kenya. Dr. Johan Brink (MSU, ABSP) and Dr. Joel Cohen (ISNAR) acted as key resource persons. The overall purpose of the meeting was to discuss the findings of a consultant's report on "*Agricultural Biotechnology in the ASARECA Region: Priorities for Research*" (submitted by MSU). The consultant's report focused on the development of transgenic crop applications. It recognized that other applications of biotechnology could be extremely valuable, especially in the African environment and also in the ASARECA region. However, they may not add value to what already falls within programs of some ASARECA member countries. The consultant therefore recommended that ASARECA should examine transgenic technologies that can offer immediate potential for substantial crop and yield improvement in the region. The most obvious of the available transgenic technologies would be insect resistance, virus resistance, fungal disease resistance and improved nutritional quality of foods. The report also stressed that any technology development must be linked to effective policy regimes and in this case intellectual property rights and biosafety from the outset of each project. The report identified priorities and opportunities, which could be adopted, existing research in the international community, and strategic partnerships with advanced research institutions.

Outcomes of the WG meeting:

- ◆ In addition to transgenics, the WG identified other important applications ranging from tissue culture, planting materials, molecular markers, diagnostics and livestock vaccines.
- ◆ The WG stressed the importance of the livestock sector in the region, particularly in terms of the development of vaccines and disease diagnostics.
- ◆ The main priority for crops in the region is input traits such as insect, viral and fungal diseases resistance as these relate to basic food security.

- ◆ The WG recommended that the ASARECA Committee of Directors consider and approve developing the Biotechnology Initiative into an ASARECA program, similar to ECAPAPA.
- ◆ An important output of the WG discussions at the meeting was a table including additional agricultural constraints in member countries.

2. Biotechnology and Biosafety Stakeholders Workshop, June 2002

The meeting of the ASARECA Biotechnology and Biosafety Stakeholders was held from June 17–19, 2002, at the Hilton Hotel, Nairobi, Kenya. Participants included members of the ASARECA Biotechnology Initiative Working Group, ASARECA Network Coordinators, Scientists from CGIAR Centers backstopping networks, private sector and NGO representatives from ASARECA member countries and partners. John Komen (ISNAR) and Don MacKenzie (AGBIOS biosafety expert, supported by ISNAR/MSU) acted as resource persons and facilitators in various parts of the meeting.

The purpose of the meeting was to establish a list of priorities for the use and development of plant biotechnology in the countries of ASARECA, and to define the role that the network will play in this development. The ultimate goal being to come up with a proposal to donors for the extension of support for the network and also for the development of pilot projects in the region.

Biosafety Strategy

Mrs. Muffy Koch (Innovation Biotechnologies), biosafety consultant, presented the background to discussions on biosafety, and summarized the main findings of her report on biosafety. The discussion following Mrs. Koch's presentation confirmed the need to adopt a sub-regional approach to biosafety capacity building.

Outcomes:

- Discussions resulted in a suggested framework of outputs, activities and indicators of success for ASARECA's biosafety agenda, including the setting up of a sub-regional biosafety service center, to conduct reviews and provide advice, training etc.

Biotechnology Strategy

Dr. Christopher Ngichabe, ASARECA coordinator for biotechnology and biosafety, summarized progress on designing the future biotechnology program. The meeting discussed the policy environment for biotechnology, which members agreed appeared to focus on biosafety and IPR, but also needs to be linked to overall national development objectives.

Outcomes:

- It was agreed that ASARECA should be a driving force for biotechnology and biotechnology policy in the region. Possible options for ASARECA would be to establish a short-term project addressing 2-3 priority constraints, applying available technology, or a longer-term program to develop new technologies.

3. ASARECA Biosafety Working Group Meeting, June 2002

Prior to the May stakeholders meeting, a meeting of the ASARECA Biosafety Working Group was called to receive the consultant's report on biosafety and to formulate the framework for a biosafety activity within ASARECA. The meeting was held from June 14-16 2002 at the Hilton Hotel, Nairobi, Kenya. The main objective of the meeting was to receive and consider the consultant's report on regulatory status and regional biosafety regulatory mechanisms and administration under ASARECA. Don MacKenzie (AGBIOS biosafety expert, supported by ISNAR/MSU) acted as a resource person throughout the meeting. John Komen (ISNAR) attended the wrap-up and concluding sessions, which helped finalize the ASARECA biosafety framework.

Don Mackenzie (AGBIOS) presented the ISNAR paper on “*Developing Biosafety Systems: A conceptual framework, critical decision points and required information.*” Muffy Koch (Innovation Biotechnologies) presented the commissioned report on “*Regulatory status and regional biosafety regulatory mechanisms and administration under ASARECA*”, which was accepted by the WG. Dr. Adiel Mbabu, ASARECA planning officer, presented the consultative framework being formulated for ASARECA.

Outcomes:

- ASARECA will run a biosafety project parallel to the biotechnology project to ensure that GMOs are developed, tested and released safely.
- ASARECA should establish a sub-regional biosafety service centre
- The roles of ASARECA will be at four levels:
 - Nationally to assist member countries to establish biosafety frameworks;
 - Sub-regionally to coordinate regulator training; Coordinate scientist training; Provide a platform for food and feed safety checks on GM commodities; Co-ordinate regional biosafety research; Provide a platform to consult on setting up a sub-regional biosafety support service; Provide a Sub-regional information service; Administration of ‘regional’ applications; Conduct Sub-regional biosafety reviews; Sub-regional biosafety research;
 - Regionally, to promote a biosafety institutional structure at the African Union (AU)
 - Internationally to interact with international players; Provide a strong Eastern & Central African voice; Source funding to carry out biosafety research.
- The frame-work for the biosafety project will be as follows:
 - Goal: The safe application of biotechnology for enhanced and sustainable productivity, competitiveness, and value-added agricultural systems;
 - Purpose: An effective and efficient sub-regional biosafety system established
 - Intermediate Results: 1). A sub-regional knowledge and skills base sufficient to support biosafety decision-making in ASARECA member countries established 2). Sub-regional templates for conducting biosafety reviews established and utilized, 3). Sub-regional biosafety policy dialogue enhanced

4. Biosafety Stakeholders Workshop, August 2002

A meeting to allow for a broader review of the findings and recommendations from the June biosafety WG meeting was held on August 1-3, 2002, in Nairobi, Kenya. ISNAR provided guidance prior to the meeting, working jointly with the ASARECA Biotechnology and Biosafety Coordinator on the key documents tabled at this meeting.

Outcomes:

- The stakeholders discussed the findings and recommendations of the biosafety Working Group and agreed to adopt and endorse the report. No amendments or additional recommendations were made.
- The stakeholders worked in groups targeting the various sections of the proposal from Introduction to Budget. The refined sections were put together and presented to plenary. This exercise took most of the time available to the stakeholders and by the third day the report, including sections on timelines and budgets, to the Committee of Directors and the Proposal had been re-written.

5. Meetings with ASARECA Coordinator for Biotechnology and Biosafety, August-September 2002

Findings from the consultants' reports, WG meetings and stakeholder meetings were incorporated in a full ASARECA program proposal, to be submitted to USAID. Joel Cohen (ISNAR) organized a review meeting in Washington DC, August 19-21, 2002, involving ASARECA, MSU and USAID. The objectives of the meeting were to:

Review the status of reports completed to date;

- Determine a strategy for the ASARECA initiative on biotechnology and biosafety;
- Define the next steps for ASARECA proposal development, incorporating comments from USAID officers.

Outcomes:

- The meeting reached agreement among ASARECA, USAID, MSU and ISNAR regarding the way forward and division of responsibilities toward completing the ASARECA program proposal on biotechnology and biosafety

6. ASARECA Working Group Meeting, September 2002 – postponed to February 2003

A final WG meeting was planned to complete the proposal-writing process. Originally scheduled for September 2002, the meeting was postponed several times for various reasons. It eventually took place on February 16-22, in Nairobi, Kenya. The final dates for the meeting allowed for a detailed review and incorporation of USAID's comments on an earlier version of the ASARECA proposal, which was the main purpose of the meeting. José Falck Zepeda (ISNAR) supported the WG throughout the meeting, working on various revisions to the biotechnology and biosafety components, in consultation with Mike Hall (USAID-REDSO). This consultative process continued after the meeting.

Outcomes:

- Revised ASARECA proposal on biotechnology and biosafety, incorporating USAID's perspectives.

7. Follow-on activities beyond the contract end date:

ISNAR has retained its commitment to seeing this process through, despite the many changes, revisions and restructuring of both the biotechnology stakeholder's priority setting, and the final results of the biosafety program consultations. In the most recent meeting of the ASARECA Working Group, held in May 2003, Donald MacKenzie again represented ISNAR and MSU in this process. In this meeting, the Working Group made further refinements to the overall framework for ASARECA's biotechnology and biosafety initiative, and came up with a final scoring of priorities.

Most recent interactions took place in September 2003 involving Dr. Cohen, Dr. Sefu Ketema (ASARECA Executive Director), Dr. Christopher Ngichabe, Dr. Theresa Sengooba (NARO, Uganda), and Dr. Mike Hall (USAID-REDSO) at ASARECA Headquarters to review the current status of all activities. While delays and complications were experienced due to the ASARECA process, addition of new stakeholders and partners, the report is still making progress while changing greatly to reflect greater precision, closer links to the ASARECA networks, and more achievable and relevant biosafety activities. A final proposal is being worked on now, including clear links to, and areas of support from the ISNAR-led Program for Biosafety Systems (PBS) and the Agricultural Biotechnology Support Project (ABSP-II) managed by Cornell University.

8. Submit final proposal

As a result of follow-on activities in 2003, the final draft ASARECA proposal will be shared with the ASARECA Committee of Directors in October 2003. Dr. Theresa Sengooba will represent the PBS Consortium. Once this is approved, the final and approved proposal will come into action in 2004, with workplans structured between PBS and ABSPII.

ISNAR continues to support final program development and submission. While not possible to attain a final proposal in the original terms of the agreement, continuity has been kept among all partners, with final process and steps described above in section 6.

It is expected that ISNAR, through the new Program on Biosafety Systems, will remain a constant partner to ASARECA, helping to implement its program on biosafety. Presently, Theresa Sengooba and Christopher Ngichabe are working on that agreement. Thus, while final proposal and workplans are still in development, it can now be said they are in their final stages, and workplans already being generated to assure continuity as per biosafety activities.

Assessment of Impacts

- ◆ During the collaborative program between ASARECA, MSU and ISNAR regarding the biotechnology and biosafety initiative, it has become clear that great progress was made with limited financial resources. ISNAR's role was primarily one of technical assistance and backstopping, which contributed to keeping the proposal drafting process moving, as summarized above.
- ◆ Following ISNAR's initial support to ASARECA in 1999, supported by UNDP, and after a time gap of almost 2 years, the ASARECA Biotechnology and Biosafety Working Group convened for 3 WG meetings and in 2 Stakeholder workshops during the period covered in this report.
- ◆ Valuable information was extracted from the consultants' reports on biotechnology and biosafety that enabled the WG to prioritize potential biotechnology interventions applicable to the ASARECA region and to gain insight on potential regional biosafety frameworks.
- ◆ Stakeholders from 10 ASARECA countries participated in 2 meetings and assisted ASARECA in the dialogue and development of a strategy for biotechnology and biosafety in the region, resulting in a high degree of regional ownership.
- ◆ ASARECA's link with ISNAR also resulted in well established relations with expert organizations in biotechnology and biosafety, such as AGBIOS. In addition, linkages with other regional players, such as BIO-EARN and ABSF, were considerably strengthened.
- ◆ Through this participatory process, an ASARECA Biotechnology and Biosafety proposal was completed and submitted to a major donor organization.
- ◆ Communications and collaboration have continued in 2003, which will among other things result in a prominent role for ASARECA in the implementation of USAID supported programs such as PBS. Such sustained commitments are a testament to the impact, appreciation, and desire for continuity achieved between ASARECA and ISNAR.

Southern African Regional Biosafety Program (SARB) Final Technical Report: Oct 2000 – June 2003

Reporting period: 1999 - 2003

Lead Principal Investigator

GJ Thompson, ARC-Roodeplaat, South Africa

Project partners

PM Koch, Golden Genomics, South Africa

Overall Project Goal

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

Project Justification

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

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

The current status of Biosafety in the SADC region

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

Project Objectives

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

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

Activity 2: Regional Workshop on Biosafety: This workshop was to serve as a general awareness-raising event on biosafety in the SADC region. It was targeted towards legislators/policy makers, regulators, members of biosafety committees as well as delegates to the Cartagena Protocol on Biosafety and Codex. 4. **Activity 3: Regional Biosafety Training Course:** The purpose of this training course was to train regulators and reviewers (preferably not previously trained) in biotechnology and biosafety issues.

Activity 4: Journalists/Media Course: Media reporting on biotechnology is increasingly influencing how policy makers develop and implement biosafety regulations as well as public perceptions regarding biotechnology. The purpose of this workshop was to provide balanced information on biotechnology and biosafety to key media in target countries. It was also to address issues of how policy makers/regulators convey issues of safety and regulation to the media.

Activity 5: National Follow-up/In-country Biosafety Training: Pending funding levels and progress by target countries in discussion of regional policy cooperation, national-level biosafety training will be held as a subset of the target countries. The purpose of these national biosafety training activities will be to broaden the range of policy makers with biosafety training to include all members of National Biosafety Committees and other stakeholders such as Ministries of trade, industry, farmers organizations, etc. National Biosafety training courses will be presented in the 7 Core Target countries. These courses will vary to meet the specific needs of each country.

Activity 6: Risk Assessment Research-Sorghum Gene Flow Case Study: A critical component of biosafety risk assessment and management will be knowledge about environmental risks specific in the region, such as gene flow from biotech crops to related African species. Gene flow studies on Sorghum will be conducted by VOPI on their research farm near Pretoria and possibly in another country to gain risk assessment data and illustrate risk management policy options. The results will provide very valuable information for the African continent.

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

Project Progress

SARB has accomplished all its objectives and greatly contributed to increased awareness of biosafety within the SADC region. All its activities have resulted in a core of people in each participating country trained in the basics of biosafety risk assessment. This has contributed towards the progress most countries have made in developing biosafety regulatory frameworks. A regional network has been established enabling communication and exchanges between countries. The impact of SARB was established by the Working Group and a report is given in Addendum 8.

Activity 1: Working Group

- ✓ One of the major accomplishments of SARB was the establishment of a regional Working Group (WG) following the first meeting in Pretoria, South Africa on 19 and 20 November 2000 (see list of current working group members in Addendum 1). In order to maintain a

connection between members of the WG and improve communication a regular newsletter was distributed. This proved to be very successful. A second meeting of the Working Group was held in Pretoria from 19 to 20 December 2002. The main objectives of the meeting were to receive progress reports from each country, determine future training needs and discuss ways of assessing the impact of SARB. The minutes of this meeting is contained in Addendum 2. A final meeting of the Working Group was held on 11 June 2003 following the Symposium. This meeting mainly dealt with establishing the impacts of SARB and discussing approaches to the future. The minutes of the last two meetings are contained in Addendum 2. The WG has formed the basis of a successful network within the SADC region and any future activities should use this foundation.

- ✓ As part of the final WG meeting a symposium “Strengthening Biosafety Capacity for Development” was held on 9-10 June 2003 near Pretoria. This symposium was organised to review the impact of SARB and other ABSP programmes. It was attended by 38 delegates from both target and non-target countries as well as representatives from other international programmes such as PBS. See Addendum 3.

Activity 2: Region Biosafety Workshop

- ✓ A key success factor for Activity 2 was the nomination of delegates who would participate in the Regional Biosafety Workshop. A total of 8 Southern African countries were visited during the period 28 January to 28 February 2001 by Dr Brink and Ms Koch. In each country, meetings were held with officials of government departments such as Agriculture, Environment as well as with Biosafety regulators (if available) and members of Biosafety committees.
- ✓ A highly successful workshop was held at the ARC Central Office in Pretoria from 28-31 March 2001, attended by 37 delegates from 11 countries.

Activity 3: Regional Biosafety Risk Assessment Workshop

- ✓ This course was held from 4 – 10 November 2001 at the ARC Central Office. A total of 24 delegates from the seven core countries representing regulators and reviewers not previously trained, attended the course.
- ✓ The course allowed for the first practical evaluation of the new ABSP Workbook for Technical Training: Biosafety and Risk Assessment in Agricultural Biotechnology. The overall opinion was that the course was very successful and the Workbook very applicable. The Workbook has subsequently been used as the basis of in-country training of scientists.
- ✓ Two further regional courses were held. The one from 12-14 February 2003 in Pretoria was attended by 29 delegates mainly from non-target countries (Botswana, Lesotho, Swaziland and Seychelles) as well as from RSA, Malawi, Namibia, Zambia and Zimbabwe. The second was also held in Pretoria from 12-14 May 2003 and attended 21 scientists from Malawi, Namibia and Zimbabwe. A report on these activities is given in Addendum 5.

Activity 4: Journalists/ Media Workshop

- ✓ The workshop was held at the ARC Central Office from 21 – 23 May 2001. This coincided with the CGIAR mid term meeting held in Durban, South Africa. A total of 17 journalists from

seven countries attended the workshop. This activity was combined with an AfricaBio/USDA training course for scientists and policy makers on how to interact and communicate with the media.

Activity 5: National In-country Biosafety Training

- ✓ The Working Group teams of each core country submitted work plans during 2001. During the second WG meeting in December 2002 each country presented its priorities for further training. In consultation with the Working Group, the plans were revised by the SARB management team and schedules drawn up.
- ✓ SARB granted Namibia financial support to test and verify their proposed biosafety regulations through the drafting of three “applications” for submission to and evaluation by their National Biosafety Forum. This was undertaken under the guidance of Ms Muffy Koch.
- ✓ Most of the training as requested by individual countries was successfully conducted. These are given in Table 1. Reports on the courses conducted between January 2002 and June 2003 are given in Addendum 4.
- x The planned two courses to be conducted in Zimbabwe during late November 2002 did not take place at the request of USAID. The one course would have been on risk assessment for scientists and the other for inspectors. Instead scientists from Zimbabwe attended the third regional course.
- x Two countries were unable to complete the second round of training. It was found that the cost to conduct a survey of public opinion in Mauritius was greater than the amount available from SARB. South Africa was unable to organize the proposed meeting between applicants and the regulatory bodies due to the public debate on the GM Act.

Table 1: Biosafety training conducted under SARB

COUNTRY	DATE	TYPE OF COURSE	NUMBER OF PARTICIPANTS	VENUE
Regional	28-31.03.01	Biosafety	37	Pretoria
Regional	22-23.05.01	Media	17	Pretoria
Regional	4-10.11.01	Risk Assessment	24	Pretoria
Regional (non-target)	12-14.02.03	Risk Assessment	29	Pretoria
Regional	12-14.05.03	Risk Assessment	21	Pretoria
Malawi	13-16.11.01	Media	25	Lilongwe
		Policy makers - awareness	25	
		Risk Assessment	30	
	28-30.03.03	Regulatory Framework – Policy makers	25	
		Regulatory Framework – scientists	21	
Mauritius	28.10-01.11.01	Risk Assessment	33	MSIRI
		International Implications	30	
		Public Awareness	42	
Mozambique	6-7.12.01	Biotechnology and Biosafety Awareness	56	Maputo
	1-4.06.03	Risk Assessment	30	Bilene
South Africa	1-8.10.02	Risk Assessment	28	Pretoria
		Regulatory council	8	
		Inspectors – risk assessment	21	
Zambia	27-31.05.02	Policy makers - awareness	15	Lusaka
		Media	23	
		Risk assessment	23	
	18-20.05.03	Advanced risk assessment	23	

Activity 6: Sorghum Gene Flow Case Study

- ✓ Initially the project was to be led by Ms M Koch but later it was decided that Mr G Bothma would take over responsibility.
- ✓ No suitable GMO sorghum could be located. Thus following discussions with sorghum breeders at the ARC Grain Crops Institute and private seed companies, trials were designed incorporating conventional seed to measure gene flow.

- ✓ A trail to monitor pollen dispersion was planted at the ARC-Roodeplaat in December 2002. It was found that pollination rate dropped off significantly with distance from the pollen source with very little pollination occurring beyond 200m.
- ✓ The second trial was conducted by Dr E. Mwenje of NUST in Bulawayo, Zimbabwe, in collaboration with ICRISAT. Using two cultivars with different seed colour it was shown that hybridization does occur when planted within a short distance of each other.
- ✓ A full report is given in Addendum 6.

Activity 7: Core Group Biotechnology Field Trip

- ✓ The tour to China to review biotechnology and biosafety in another developing country was organized with the assistance of a local company, China Experience Trade & Tours Company (Pty.) Ltd.
- ✓ The tour took place from 7 to 15 August 2002 with fifteen delegates from the seven countries. They were accompanied by Dr. G. Thompson and Mr. G. Bothma of the ARC-Roodeplaat and Dr. L. Heunis of China Experience.
- ✓ Participants gained insight into the regulatory process and biotechnology research in China which initially had not placed much attention on biosafety of the GM crops developed. It had now adopted a more precautionary approach and implemented a system of biosafety risk assessment. The benefits of Bt cotton were expounded to the delegates.
- ✓ A full report of the tour is given in Addendum 7 (*available on request*).

Summary Of Achievements And Constraints

Achievements

1. More than 600 people sensitised to biosafety within the region.
2. 380 scientists trained in the basics of conducting a risk assessment of GM crops or products.
3. A regional network established through which biosafety issues can be promoted and deliberated.
4. Three countries now have legislation on GMOs while another three have draft legislation due to be presented to their respective parliaments shortly. The last country, Mozambique, has instituted regulations for dealing with GM food aid and has begun the process of drafting legislation. SARB has contributed towards all these processes and improving the implementation of regulations in those countries which already had legislation.
5. SARB contributed greatly to the heightened awareness of GMO technologies and the safety thereof within the region. This was mainly through its training of journalists and conducting workshops for policy makers and public.

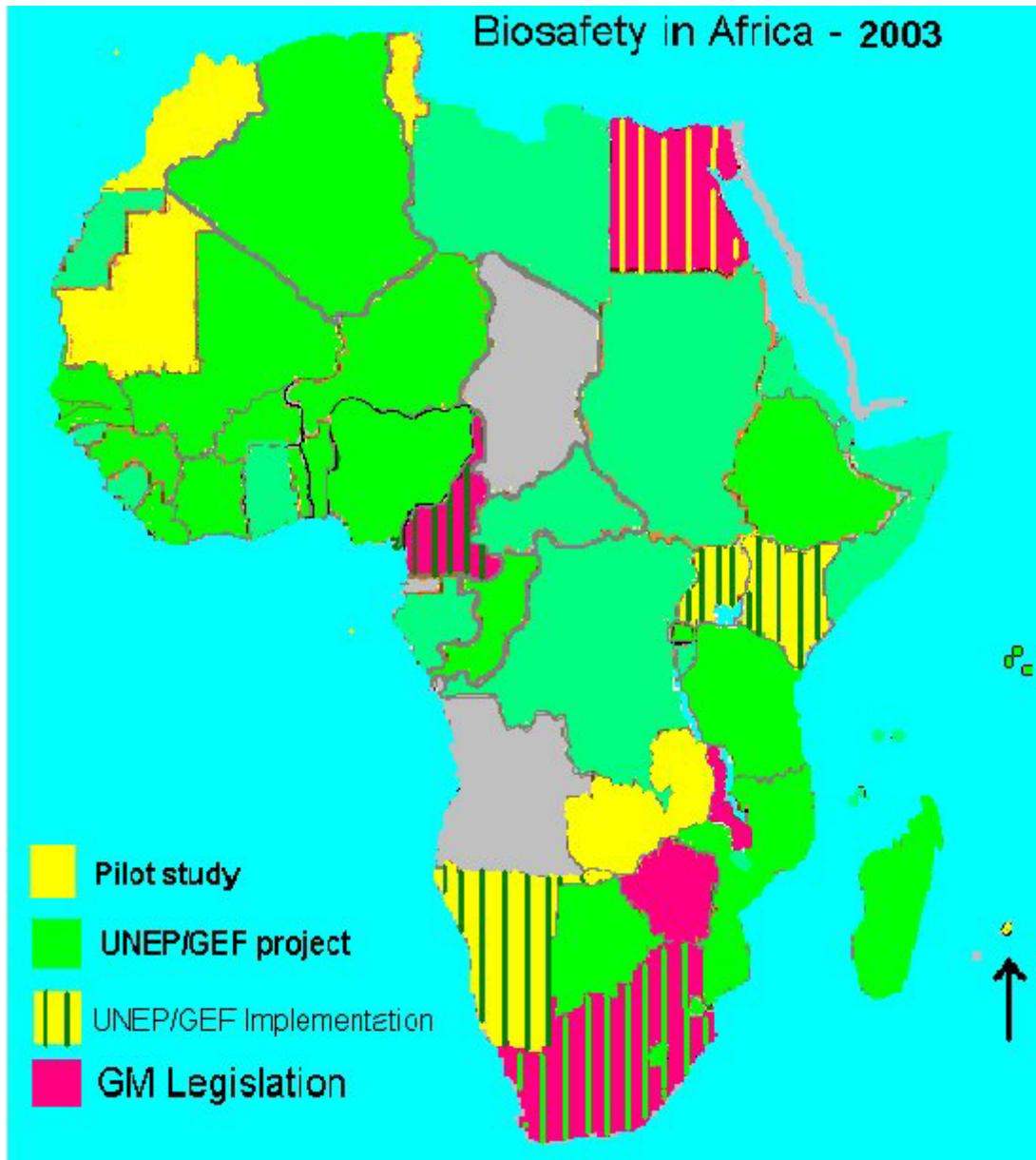
6. The greatest success lay in the in-country training whereby each country through its Working Group members, established its own training needs, developed its own work plan and assisted SARB in executing it.

Constraints

1. The political support for the programme was not as great as we would have liked it to be in some countries.
2. A programme of this size required a full time coordinator. Due to the change in management at ARC-Roodeplaat this was not feasible.
3. In that members of the Working Group were performing tasks for SARB on a part time basis it was often difficult for them fulfil obligations on time. This was compounded by the different members being situated in different departments or institutions.
4. Communication was a problem at times but this was overcome through the creation of a newsletter.

[Full Report with Appendices Available on Request]

Figure 1: Status of Biosafety in Africa in 2002



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ABSP Country Reports



Biotechnology Research and Policy Activities of ABSP In Egypt

1991--2002



The Agricultural Biotechnology Support Project

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ABSP Biotechnology Research and Policy Activities in Egypt

1991-2002

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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 such 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.

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 2 years worth of data on the resistance of transgenic *Spunta* potatoes, a local fresh market cultivar in Egypt. These *Spunta* lines, transformed with a *cryV* 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 is unclear how Egypt will develop its commercialization procedures for transgenic potatoes, but if

it adopts a stringent European model, it will be difficult for a public-funded effort to meet the regulatory costs.

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.

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Development of virus resistant cucurbits

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

1995-2003

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 Raket did not regenerate well in the *Agrobacterium* system. An MSU international graduate student from Egypt 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 R₂ 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.

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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 are:

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.

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 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.

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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.

Egypt Biosafety

Activities

- ◆ **Biosafety Internship Program: Guidelines Development, MSU, May-July 1993.**
 ABSP organized an eight week internship 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, Kenya and Indonesia participated in the ABSP Biosafety Intern Program, May-June 1993. The Indonesian scientists then participated in a *hands-on* biosafety training program at ICI Seeds, Iowa, while the Egyptian and Kenyan Scientists participated in a program at Michigan State University. The scientists then reconvened in Washington DC where they had the opportunity to interact with federal personnel at USDA/APHIS, FDA and EPA responsible for various aspects of biosafety.
- ◆ **Genetic Resources Workshop, Egypt, June 1994.**
 The ABSP/AGERI project, in cooperation with Genetic Resources Communication Systems (GRCS), Inc., the Egyptian National Research Program, and USAID/Cairo held a two day Genetic Resources Workshop in Cairo. The workshop brought together experts from Egypt and from the international Community to discuss various issues related to genetic resources in Egypt and the region. A special Issue of *Diversity* journal focusing on this workshop was published. The Mediterranean issue of *Diversity* was translated into Arabic and 3,000 copies of this version were distributed.
- ◆ **Biosafety Workshop at AGERI, Egypt, January 1994.**
 The goal of this workshop was to create a greater awareness and strengthen the biosafety regulatory framework in Egypt and the Middle East. The workshop involved international experts on in biosafety, and scientists and regulatory personnel from Egypt and selected countries in Africa. The workshop addressed policy, risk assessment and field-testing issues surrounding the management and safe handling of transgenic plants. The proceedings were published [*Biosafety/Intellectual Property Rights Project Evaluation, Proceedings from the AGERI & ABSP Workshop Series January 24 - 31, 1994, Cairo, Egypt.*]
- ◆ **Consultations on Egyptian Biosafety Guidelines, 1994.**
 Dr Patricia Traynor reviewed the Egyptian draft biosafety guidelines and provided comments.
- ◆ **Construction of Containment Greenhouse Facility at AGERI, Egypt, 1995.**
 ABSP provided leadership in the development of a cooperative sub agreement 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 who stated in his report that “the biocontainment greenhouse facility at AGERI meets the international standards for growing genetically engineered organisms, and is ready for commission.”

Impacts

◆ Biosafety Guidelines Developed and Approved

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. Impacts of the ABSP have included:

- Training of the AGERI Biosafety Officer assigned with drafting biosafety guidelines for laboratory, greenhouse and field experiments
- Construction of a biocontainment greenhouse facility
- 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 were established in 1998 by Ministerial Decree No. 1648. Development of food safety laws/regulations is currently underway.
- Field-testing of GMOs, including insect-resistant potatoes (MSU and AGERI), virus resistant squash (AGERI) and virus resistant tomatoes (AGERI) and Bt resistant maize (Fine Seeds/Novartis).

Egypt IPR/Technology Transfer

Activities

- ◆ **Intellectual Property/Patent Internship Program, Stanford University, April 1993.**
An IPR internship program was designed and implemented by Professor John Barton of Stanford Law School from April 1-30, 1993. Seven interns from Egypt, Kenya and Indonesia participated in the program, the goal of which was to provide hands-on experience to legal and scientific personnel from developing countries in various issues related to intellectual property rights. In addition the internship enhanced communication between those involved in the sciences and those with responsibilities in the legal issues surrounding biotechnology. The program encouraged the assessment of current intellectual property structures within the participants' home countries, provided access to literature and expertise regarding IPR in both the public and private sectors.
- ◆ **Workshop On Intellectual Property Rights, Patents & Licensing, Egypt January 1994.**
This workshop, designed by Prof. John Barton, George E. Osborne Professor of Law at Stanford Law School, was held in Cairo, Egypt from January 24-25, 1994. Over 100 participants from various public and private sector institutions attended the workshop, 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. Proceedings of this workshop were published.
- ◆ **IPR Workshop, Washington DC, July 1994.**
ABSP sponsored this workshop in Washington DC from July 11-14, 1994 as a follow up to the Egypt workshop. Forty-four participants attended from Egypt, Kenya, Indonesia and Costa Rica, Thailand, Sri Lanka as well as a number of institutions and agencies such as USAID and the World Bank. The purpose of the workshop was to present intellectual property rights in biotechnology as an important issue to institutions and individuals. Proceedings of this workshop were published: *Intellectual Property Rights, Proceedings from the ABSP Workshop Series July 11 - 14, 1994, Washington, D.C.*
- ◆ **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 (TT) 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 Indonesia (8), Costa Rica (1), South Africa (2), Egypt (5), Morocco (5), and Kenya (2) 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. ABSP partner countries displayed information about their TT offices and the technologies that are available for licensing.

◆ **ABSP Industrial Seminar Series**

In April 1993, the ABSP organized an Industrial Seminar Series (ISS). The ISS was organized to provide opportunities for senior scientists and administrators from the public and private sector, and government officials from the ABSP partner countries (Costa Rica, Egypt, Indonesia, Kenya) to interact with technical and business personnel at private biotechnology companies in the U.S. that have active agricultural biotechnology programs. In addition to seven participants from the ABSP partner countries, three participants from Jamaica (sponsored by the USAID-Jamaica) also attended the ISS. The companies visited included Garst/ICI Seeds, Inc. (now Syngenta), Ecogen Inc (now part of Monsanto), and DNA Plant Technology. The ISS was instrumental in opening lines of communication between developing country leaders and host companies. It also provided participants an exposure to a diverse group of companies oriented towards different end-user groups.

IPR/PVP Workshop, Michigan State University, East Lansing, Michigan, September 2001.

Dr. Fred Erbisch, ABSP consultant, spent three weeks, Summer 2001, assisting the Agricultural Research Center (ARC) in developing basic materials and policy for the planned technology transfer office. As a result of this consultation, Drs. Erbisch and Maredia developed two workshops at MSU for ARC participants. Participants of the IP Management Workshop will operate a technology transfer office in Egypt that the ARC will establish. Participants of the Plant Variety Protection (PVP) Workshop will staff a new Egyptian PVP Office.

Impacts

◆ **Establishment of the Technology Transfer Office at Agricultural Genetic Engineering Research Institute (AGERI).**

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 a 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).**

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 first 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.**

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.

Egypt Assessments

◆ **AGERI, Giza, Egypt—3-year project agreement between Haas School of Business and ABSP.**

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.

◆ **AGERI: Strategic Marketing Plan, 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; 8) 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.**

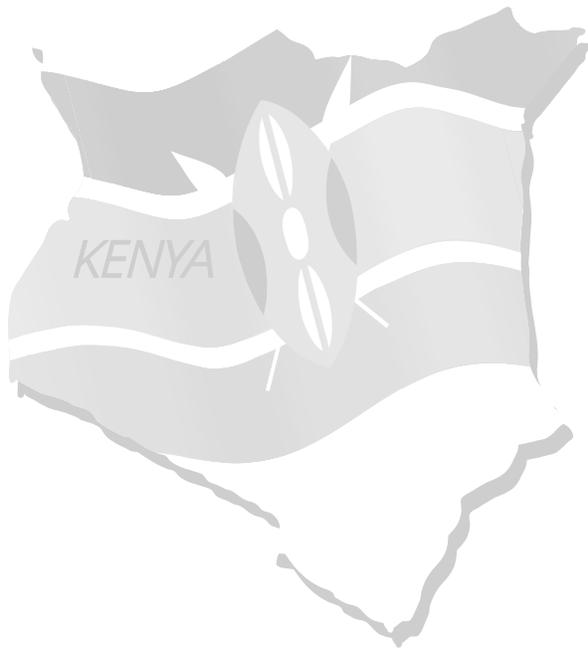
The third Haas team investigated prospects related to AGERI's internal organization. The assessment was again in two phases that included: 1) preliminary research in the USA

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

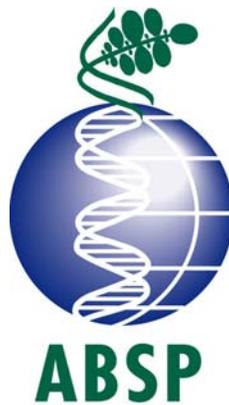
◆ Training at MSU

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, Director, 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 bioinformadics at AGERI. The new equipment is being shipped to Egypt in the near future. 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.



Biotechnology Research and Policy Activities of ABSP In Kenya

1991-2002



The Agricultural Biotechnology Support Project

Supported by the United States Agency for International Development (USAID) and implemented by Michigan State University under Cooperative Agreement: DAN-A-00-91-00126-00

ABSP Biotechnology Research and Policy Activities In Kenya

1991--2002

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Screening for insect resistance in Kenyan maize

Kenya Agricultural Research Institute (KARI), Kenya
CIMMYT, Kenya

1997-2000

Project Goals

1. To collect local and exotic maize germplasm for use in screening for resistance to Lepidopterous stemborers

To elucidate the genetic basis of host plant resistance against *Busseola fusca* and *Chilo partellus* by use of artificial infestation

To develop a heterotic population with adequate levels of resistance to stemborers

Lepidopterous stemborers constitute the most widely distributed and serious group of insects attacking maize in Kenya. About 23 stemborer species have been recorded. Amongst them, the most common stemborers are in the spotted stemborer family (SSB), including *Chilo partellus*, *C. orichalcillielus* and *Eldana sacharina*, Walker. In the *Noctuodae* family are the maize stemborer (MSB) *Busseola fusca* Fuller and *Sesamia calamists* (pink stemborer).

Many strategies to reduce losses due to stemborers, estimated at 23-53%, have been practiced. These include chemical pesticides, cultural and other management practices, including early planting. However, chemical control is not very effective and development of maize varieties with host plant resistance (HPR) is generally considered the most cost-effective method for controlling insect damage in a sustainable agricultural systems. Development of multigenic resistance to stem borer was the main goal of this study.

Project Impacts

Since 1997, 51 local and exotic lines have been collected and planted during the long rainy season. In addition, lines have been obtained from CIMMYT and Cape Town. Of the lines screened for resistance to infestation by *Busseola* and *Chilo*, only two inbred lines showed good tolerance against both *Chilo* and *Busseola* infestation.

In 1998, new sources of resistance were examined in 127 maize lines planted at the Embu main station. Two inbred lines showed acceptable tolerance to both SSB and MSB under artificial infestation.

In 1999, 28 maize accession lines were evaluated. Six lines showed high tolerance to *Busseola* and *Chilo*, while other lines showed moderate tolerance. Days to flowering were not affected.

Data suggest that most of the materials tested could be selected for foliar damage tolerance mainly due to the decrease in tunnel length – a tolerance attribute. Continued work in collaboration with CIMMYT and support from other donors will elucidate the genetic basis of the tolerance.

Development of insect and virus resistance in sweetpotato

Monsanto Co., USA
Michigan State University, USA
Kenya Agriculture Research Institute (KARI), Kenya
Central Research Institute for Food Crops (CRIFC), Indonesia
International Service for the Acquisition of Agri-Biotech Applications (ISAAA), USA
International Potato Center (CIP), Peru

1992-2000

Project Goal

In Kenya the most important root and tuber crops are potato, cassava and sweetpotato, which is the most widely distributed, and as elsewhere in Africa, sweetpotato is mainly grown by women small-scale farmers. Despite the importance of sweetpotato for smallholder farmers in Kenya, there are serious production problems facing the crop, including pests and diseases and inadequate quantities of good quality planting materials. The major pests include sweetpotato weevils and vertebrate pests. Sweetpotato virus disease (SPVD) is the most important disease of sweet potato in Africa, infected plants yielding less than 50% compared to virus free plants. SPVD is caused by a dual infection with sweetpotato chlorotic stunt virus (SPCSV) and sweet potato feathery mottle virus (SPFMV). SPCSV is transmitted semi-persistently by the whitefly *Bemisia tabaci* and SPFMV is transmitted non-persistently by aphids. Many of the important early maturing and high yielding sweetpotato varieties are highly susceptible to this virus. The overall goal of the project was to develop transformed Kenyan sweetpotato varieties with resistance to Sweetpotato Feathery Mottle Virus (SPFMV) in collaboration with Monsanto, and to transfer the improved varieties to Kenya.

Specific project objectives included the following:

- i. To develop suitable assay systems for virus challenge and protection of coat protein gene transformed sweetpotato,
- ii. To train KARI scientists and technical staff in all aspects of technology development, biosafety evaluation and Intellectual Property Rights (IPR),
- iii. To prepare biosafety application and evaluation structures to enhance the transfer and field evaluation of transgenic sweetpotato in Kenya, and
- iv. To improve production of sweetpotato in Kenya through tissue culture.

Project Impacts

- ◆ One sweetpotato variety was successfully transformed using the SPFMV coat protein gene and has shown good levels of virus resistance in laboratory and glasshouse trials.

- ◆ Regulatory approvals for the field-testing of the sweet potatoes were developed and passed by the national Biosafety Committee. The transgenic sweet potatoes were one of the first products to be reviewed by the Kenyan National Biosafety Committee.
- ◆ Field trials were planted in late 2000.
- ◆ Several Kenyan scientists were trained in tissue culture techniques and in transformation technologies
- ◆ Kenyans researchers and policy makers were trained in the area of IPR and biosafety.
- ◆ Kenyan research capacity was improved, and facilities for laboratory/glasshouse and field biosafety containment were developed and/or upgraded.

This informal arrangement involving ABSP illustrates an example of research and policy collaboration, between Monsanto and the Kenyan Agricultural Research Institute (KARI). Monsanto donated the technology royalty-free for use in sweet potatoes in Africa, effectively removing any intellectual property constraints to transferring the technology to Kenya. ABSP had identified Kenya as a focus country for Africa and identified sweet potato as an important crop for both Kenya and Indonesia. ABSP also supplied Monsanto with information about technology transfer to developing countries. In the process, ABSP supported a postdoctoral researcher at Monsanto and short-term visits of Kenyan and Indonesian scientists to Monsanto. It also funded a biosafety consultant to assist Kenyan scientists in developing a proposal for review by the Kenyan Biosafety Committee and USAID's Biosafety Committee and supported a direct subagreement with KARI to assist in in-country capacity development and technology transfer. At the end of the initial grant, Monsanto continued to support the project from its own resources and from funds provided by several other organizations. ABSP also provided support and training in the setting up of 'mock' field trials in preparation for the actual trials. These biosafety capacity building activities of ABSP and other organizations, including the International Service for the Acquisition of Agri-biotech Applications (ISAAA) have contributed substantially to Kenya's leading position in sub-Saharan Africa in moving forward in the application of biotechnology.

Kenya Biosafety

Activities

- ◆ **Biosafety Internship Program: Guidelines Development, MSU, May-July 1993.**
 ABSP organized an eight week internship 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, Kenya and Indonesia participated in the ABSP Biosafety Intern Program, May-June 1993. The Indonesian scientists then participated in a *hands-on* biosafety training program at ICI Seeds, Iowa, while the Egyptian and Kenyan Scientists participated in a program at Michigan State University. The scientists then reconvened in Washington DC where they had the opportunity to interact with federal personnel at USDA/APHIS, FDA and EPA responsible for various aspects of biosafety.

- ◆ **Biosafety Workshop at AGERI, Egypt, January 1994.**
 The goal of this workshop was to create a greater awareness and strengthen the biosafety regulatory framework in Egypt and the Middle East. The workshop involved international experts on in biosafety, and scientists and regulatory personnel from Egypt and selected countries in Africa. The workshop addressed policy, risk assessment and field-testing issues surrounding the management and safe handling of transgenic plants. The proceedings were published [*Biosafety/Intellectual Property Rights Project Evaluation, Proceedings from the AGERI & ABSP Workshop Series January 24 - 31, 1994, Cairo, Egypt.*]

- ◆ **Development of Biotechnology Initiative with ASARECA, East and Central Africa, 1999-2000.**
 ABSP has entered into a formal, contractual collaboration with the Association for Strengthening Agricultural Research in Eastern and Central Africa (ASARECA). As part of the process to develop a regional initiative in biotechnology and biosafety, ASARECA established a working group (WG) to examine issues and pragmatic approaches for integration of biotechnology through the existing regional networks and for the expansion of regional biosafety regulatory policy development.
 - ◆ ABSP Technical Support. In order to assist ASARECA, ABSP will provide technical support to the WG throughout the planning process. This will ensure that the WG members have access to international expertise in agricultural biotechnology and biosafety.
 - ◆ Biotechnology Inventory. As part of the technical support process, ABSP has developed *An Inventory of Agricultural Biotechnology for the Eastern and Central Africa Region*. The draft report highlights the current status of biotechnology applied to crops within ASARECA networks in order to give the working group an indication of the future potential of biotechnology tools for the improvement of crops that are important to Africa.

Impacts

Kenya received less financial support for policy and research efforts compared to Egypt and Indonesia, primarily because USAID/Nairobi did not commit additional funds to the program. However, USAID/Africa Bureau did provide a small amount of additional funding for research and policy efforts that assisted the ABSP in providing training and support to Kenya for biosafety regulatory development. Even so, Kenya has made significant progress in the development of its regulatory system. Kenya has:

- ◆ **Instituted a National Biosafety Committee in 1996.**
The Kenyan NBC began implementation of biosafety review processes in 1997.
- ◆ **Field-tested transgenic sweet potatoes in early 2001.**
This is the first field test of a transgenic crop developed in collaboration with the public sector in Sub-Saharan Africa (excluding South Africa).

Kenya IPR/Technology Transfer

Activities

- ◆ **Intellectual Property/Patent Internship Program, Stanford University, April 1993.**
An IPR internship program was designed and implemented by Professor John Barton of Stanford Law School from April 1-30, 1993. Seven interns from Egypt, Kenya and Indonesia participated in the program, the goal of which was to provide hands-on experience to legal and scientific personnel from developing countries in various issues related to intellectual property rights. In addition the internship enhanced communication between those involved in the sciences and those with responsibilities in the legal issues surrounding biotechnology. The program encouraged the assessment of current intellectual property structures within the participants' home countries, provided access to literature and expertise regarding IPR in both the public and private sectors.
- ◆ **IPR Workshop, Washington DC, July 1994.**
ABSP sponsored this workshop in Washington DC from July 11-14, 1994 as a follow up to the Egypt workshop. Forty-four participants attended from Egypt, Kenya, Indonesia and Costa Rica, Thailand, Sri Lanka as well as a number of institutions and agencies such as USAID and the World Bank. The purpose of the workshop was to present intellectual property rights in biotechnology as an important issue to institutions and individuals. Proceedings of this workshop were published: *Intellectual Property Rights, Proceedings from the ABSP Workshop Series July 11 - 14, 1994, Washington, D.C.*
- ◆ **East Africa IPR Workshop, Uganda, 1999.**
ABSP held a workshop on *The Impact of Intellectual Property Rights on International Trade and Agriculture in East Africa* in Kampala, Uganda from January 18-20, 1999. The Ugandan Council for Science and Technology (UNCST) assisted ABSP in the local organization of the workshop. Additional funds for the support of regional participants to attend the meeting were obtained from the Technical Center for Agricultural and Rural Cooperation (CTA, Netherlands), the Rockefeller foundation and Monsanto. Over 70 participants attended the workshop from Ethiopia, Kenya, Nigeria, Tanzania, Uganda, Zambia, Zimbabwe, Switzerland, United Kingdom, France, United States, Costa Rica, South Africa, and the Netherlands.

Impacts

Kenya has also received support in IPR and Technology Management, primarily through support of Kenyan scientists to the MSU IPR course. Impacts include:

- ◆ Development of trained staff within the Kenyan Plant Breeders' Rights Registration Office (PBRR)

- ◆ The Plant Breeder's Rights Registration Office (PBRO) was established in 1997 and has received over 300 applications, of which 15 have been provisionally granted. Three-quarters of the applications are in cut flowers, but a few are on local varieties of crops.



Biotechnology Research and Policy Activities of ABSP In Indonesia 1991--2002



The Agricultural Biotechnology Support Project

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ABSP Biotechnology Research and Policy Activities In Indonesia

1991-2002

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Micropropagation of tropical crops for commercial planting (Indonesia)

Fitotek Unggul, Indonesia
DNA Plant Technology (DNAP), USA

1991-1996

Project Goal

The impetus for this project came from Fitotek Unggul, a small Indonesian tissue culture company because they were receiving large orders for pineapple plantlets for Indonesian plantations. Conventional production of pineapple, although less costly than other means, could not keep pace with the demand, and tissue culture offered a cost-effective method to provide disease-free seedlings year around. The primary goal of this project was to develop methods for pineapple micropropagation in liquid cultures, to transfer the technology to Fitotek, and to begin the process of commercialization.

Project Activities

This collaborative project capitalized on initial development of bioreactor technology for axillary shoot bud multiplication of pineapple in a liquid culture system. It was hoped that this method would be Fitotek's answer for further growth in anticipation of a demand for more than 15 million plants per year. DNAP had extensive experience in tissue culture/regeneration in a large number of plant species, including major tropical plantation crops, and Fitotek had been in the business of plant propagation for several years and was able to contribute expertise in commercial micropropagation and sales networking for the target crops. The companies cooperated successfully to maximize the benefits of advanced micropropagation methods and to actively market the products of these ventures.

Project Impacts

- ◆ In its first uses of the bioreactor for mass propagation, Fitotek was able to use 32 initial shoots to produce 3,400 harvestable plants with greater consistency of size and color.
- ◆ The bioreactor system was effective for commercial production with the potential of producing pineapple plants 40% more cheaply than previous methods. This system effectively reduced requirements for labor, raw materials, electricity and space.
- ◆ With these encouraging results, Fitotek added several bioreactor units with the capacity of producing 12 million plantlets a year. However, the demand for pineapple declined rapidly during the project, and necessitated a reassessment of this strategy. At the same time, demand for ginger in the country was rising, and Fitotek was able to adapt its micropropagation techniques to produce ginger instead of pineapple.
- ◆ This project illustrates a successful collaboration between private sector partners, and the flexibility of the partners to meet changing market demands.

Development of Asian corn borer resistance in tropical maize

Garst Seed Company (formerly ICI Seeds, Inc.), USA
Central Research Institute for Food Crops (CRIFC), Indonesia

1995-1998

Project Goals

In the U.S. the European corn borer (ECB) (*Ostrinia nubilalis*) is an important pest of maize, and maize transformation strategies using Bt genes have been highly effective in reducing losses to this insect. In Indonesia a close relative of the ECB, the Asian stem borer (ASB) (*Ostrinia furnacalis*) is the major pest. The life cycle of this insect allows it to threaten maize virtually all year round. The specific goals of this project were:

- ◆ To produce tropical maize with resistance to Asian stem borer (ASB) (*Ostrinia furnacalis*).
- ◆ Transfer of enabling technologies to Indonesian scientists via training in the US.
- ◆ Commercialization of insect resistant germplasm generated from the project.

Project Impacts

The Garst/CRIFC collaboration produced was a technical success. Achievements included:

- ◆ Optimization of a cryV gene for expression in maize
- ◆ Demonstrated mortality of the Asian Corn Borer to the cryV protein
- ◆ Development of constructs for maize transformation utilizing the codon-modified cryV gene and the maize polyubiquitin promoter
- ◆ Transformation of tropical germplasm line PN2119 and temperate hybrid line A1888xB73 using the biolistic gun
- ◆ Transformation of the temperate hybrid line A1888xB73 using Garst's "whiskers" technology
- ◆ Field testing of transgenic A188xB73 lines for efficacy against the first and second generation European Corn Borer
- ◆ Of the 248 event-plan combinations, 39 exhibited first generation corn borer resistance as determined by a visual system of leaf damage assessment

Additionally, three Indonesian scientists were trained at Garst for five weeks to learn biosafety and regulatory issues. An additional four Indonesia scientists received technical training lasting from three to 12 months in which they learned:

- ◆ Maize transformation and regeneration
- ◆ Tissue culture of tropical and temperate germplasm
- ◆ Insect bioassays
- ◆ Molecular characterization; i.e. PCR, ELISA, Southern blots, Western blots
- ◆ Field evaluation, artificial infestation and statistical design/interpretation
- ◆ Industrial research and development

However, while a success from a research and capacity building point of view, the project was not ultimately successful in developing tropical maize for use in Indonesia. This was due to primarily policy constraints, but also additional technical constraints. While initially focused on using tropical germplasm, phytosanitary restrictions forced the project to initially transform a temperate line of maize. The legal uncertainty surrounding commercialization of maize developed using the biolistic gun required the use of ICI's proprietary technology, which was only successful in transforming one particular temperate line of maize. This material would have to be backcrossed into tropical maize for development of material suitable for Indonesia. Additionally, the Bt gene, which has been incorporated into the maize, was also proprietary.

At the time of the project, Indonesia did not have in place patent or plant variety protection laws that would protect hybrid seed and transgenic plants. Indonesia still cannot provide adequate legal protection for this material, although they have recently passed a Plant Variety Protection Law. Unfortunately none of these issues were brought to the table when the initial collaboration was undertaken. In this case, partners expressed reluctance to make commitments until the results of the research were known. Thus both the scientific and training component of the project proceeded with great success, but when the scientists returned home, no mechanism existed for them to transfer to their own country the genes and varieties with which they had worked at Garst.

At that time, Indonesia also lacked the appropriate biosafety guidelines or regulations for field-testing of genetically engineered plants, and many companies, as well as the ABSP, are reluctant to test material in countries without adequate biosafety policies. National guidelines were subsequently passed by ministerial decree on September 2, 1997, and with funding from the World Bank and the Indonesian government, construction of a biosafety containment facility began that year. There are currently several field trials of transgenic crops in the country, all of which have been produced by multinational companies.

The patent laws issues in Indonesia are still largely unresolved regarding protection of genes, and if this situation does not change it will continue to inhibit public sector research institutes from accessing proprietary materials from either the public or private sectors outside the country.

Although USAID and ABSP tried to pre-empt the policy issues that would affect the technology transfer process, additional levels of unforeseen detail were encountered that brought the process to a halt. In the case of Indonesia, the biosafety issues have now largely been overcome, but the questions of IPR still have to be resolved. The transgenic material produced during the project is held in trust, however the research contract with Garst has since expired and due to budgetary constraints was not renewed.

Indonesia Small Grants Program

The Indonesian Small Grants Program was a component of the ABSP collaboration with Indonesia, supported by USAID/Jakarta. The program, which funded grants up to an equivalent of \$25,000, was open to both public and private sector institutions, and was designed to encourage the development of applied technologies of importance to Indonesian agriculture. Grants were solicited widely across Indonesian research institutes, universities and companies, and reviewed and approved by a Competitive Grants Review Committee. The Review Committee was composed of a co-ordinator from the State Minister for Scientific Research and Development based at CRIFC, and representatives from the industrial crops private sector, the horticultural crops private sector, the estate crops private sector, the university sector, the National Institute of Sciences R&D Center for Biotechnology, and the ABSP core program. The program was managed by CRIFC and the summaries below briefly outline those projects funded by the program. Total funding for the Small Grant Program was \$100,000.

Use of immunoassay probe for detection and monitoring of *Phytophthora* spp., a causal agent of pod rot of *Theobroma cacao*

Biotechnology Research Unit for Estate Crops, Indonesia

Project Goal

Cocoa is an important crop in Indonesia with more than 58% of the 221,000-ton total production coming from smallholdings. The existing plant materials in Indonesia and other cocoa producing countries are susceptible to pod rot disease caused by *Phytophthora* spp. Forty percent of pod loss is usually from damage due to the disease and chemical control is currently the major control strategy. Fungal propagules in the soil are the primary cause of infection, but there are no techniques available to quantify the level of inoculum in the soil. Immunoassay has the potential to detect and quantify the pathogen population in the soil.

The objectives of this project were as follows:

1. To investigate if *Phytophthora* associated with cacao in Indonesia consists of several serotypes.
2. To produce polyclonal antibodies (PcAbs) against *Phytophthora* spp. associated with cocoa pod rot disease.
3. To investigate the potential of these antibodies for assessing inoculum potential of *Phytophthora* spp. in soil

Project Impacts

- ◆ The polyclonal antibody developed in this study was highly specific to the *Phytophthora* species associated with pod rot disease of cacao and did not cross react to other soil microorganisms.
- ◆ The antibody was further characterized and found to be bound to polysaccharides with molecular weights of 36, 25, 20 and 17 KDa.
- ◆ Dot Blot Immunosorbent Assay (DIBA) was determined to be an improved method for the detection and monitoring of *P. palmivora*, causal agent of pod rot disease of cacao.

Regeneration study of Indonesian sweet potato

Research Institute for Food Crops Biotechnology (CRIFC), Indonesia

Project Goal

Problems in sweet potato production in Indonesia are caused mainly by sweet potato weevil, and conventional breeding methods have so far failed to produce resistant material. The application of recombinant DNA technology therefore has potential to address this problem, however, this has been hampered due to the inability to regenerate plants efficiently.

The objective of this project was therefore:

- ◆ To study the *in vitro* culture ability and regeneration of seven Indonesian sweet potato cultivars.

Ginger micropropagation using bioreactor system

PT Fitotek Unggul, Indonesia

Project Goal

Ginger (*Zingiber officinale* Rose) is a herbaceous plant indigenous to Indonesia and widely spread throughout India and China. Ginger rhizomes are used as spices, in herbal medicine, and as raw material in the food, beverage and pharmaceutical industries. The demand for fresh and dry ginger and its essential oil on the world market is high both in domestic and international trade. Ginger is propagated through rhizomes and farmers usually take planting material from their own product, a practice that tends to spread diseases. Micropropagation methods have been developed for ginger and have made possible the supply of disease-free material year round. However, solid culture systems are limited in their ability to produce material in bulk, and therefore a bioreactor system of agitated liquid culture is recommended.

The main objective of this project was:

- ◆ To investigate the feasibility of producing ginger using a micropropagation bioreactor system.

The development of CVPD free citrus seedling from protoplast fusion and embryogenic callus cultures

Gadjah Mada University, Indonesia

Project Goal

Citrus greening disease is a major cause of crop and tree loss in many parts of Asia and Africa. Before it was identified as one disease, it was known by various names: yellow shoot (huanglungbin) in China; likubin (decline) in Taiwan; dieback in India; leaf mottle in the Philippines; citrus vein phloem degeneration ((CVPD) in Indonesia; and yellow branch, blotchy-mottle, or greening in South Africa. As it became clear that all these were similar diseases the name "greening" has been widely adopted. Losses due to greening are not easy to assess but are high in many citrus growing areas. Sometimes only sectors of a tree are affected and losses are small, but in other cases the entire tree is infected and crop loss is total. No detailed loss studies have been published, but in Indonesia not less than 3 million trees were destroyed between 1960 and 1970, with groves in most regions of Java and Sumatra being abandoned by 1983.

Greening is caused by an unculturable Gram-negative phloem limited bacteria belonging to the alpha subdivision of the *Proteobacteriaceae*. The 16S rDNA comparative studies led to the proposed classification of the causal agent as a "*Candidatus*" with generic name *Liberobacter*, as defined for uncultured organisms. Two distinct species have been identified based on sequence comparison and the names, *Liberobacter africanum* and *Liberobacter asiaticum*, have been proposed for the African and Asian greening organism respectively.

The objective of this project was to:

- ◆ Use tissue culture techniques to obtain citrus seedlings free of the CVPD agent.

Project Impacts

- ◆ The plant material that gave the best explant material for embryogenic nucellus cell culture was determined to be immature fruits.
- ◆ Optimal culture media for callus culture and regeneration were identified for several citrus varieties.

Further funding for this project was obtained from The Indonesian Directorate General of High Education.

Indonesia Biosafety

Activities

◆ Public Awareness/Acceptance of Biotechnology in Indonesia

Over the past five to six years, ABSP has assisted a number of CRIFC faculty and staff in both biotechnology technical training and in regulatory and intellectual property policy training. This training has provided the researchers at CRIFC with sound scientific knowledge to engage in current biotechnology education and awareness campaigns throughout Indonesia. Teams of scientists recently traveled in Central Java, West Java and Bali to present seminars on the genetic engineering for crop improvement and Indonesia's regulatory system for GMOs. The audience was primarily university and research institute personnel. CRIFC plans to expand this effort to include East Java and will conduct training to educate additional scientists on communicating biotechnology issues and policy to the Indonesian public.

◆ Assistance to Indonesia in Developing National Biosafety Guidelines

Indonesia has been a major focus country for ABSP's capacity building in biosafety and intellectual property rights. In 1995, ABSP began providing Indonesia with assistance in developing its national biosafety guidelines.

- ◆ A consultant from the USDA National Biological Impact Assessment Program worked as a special consultant for ABSP and assisted the committee formed by CRIFC in drafting the guidelines. Indonesian experts in each of three research sectors (plants, animals, and microorganisms) were selected as the writing committee with the approval of Indonesia's Ministry of Agriculture. A first draft was produced and entitled *"Guidelines for Planned Introductions into the Environment of Organisms Genetically Modified by Recombinant DNA Techniques."*
- ◆ In order to improve upon this first draft, CRIFC and ABSP organized a biosafety workshop, held in May 1996, and a total of 45 participants from both the public and private sector attended. Based on the workshop, a new draft was produced, and the guidelines for biosafety were proposed as the basis of a decree from the Minister of Agriculture.
- ◆ A second workshop was then held to finalize the second draft, which was reviewed by Indonesian officials and the Bureau of Law at the Indonesian Ministry of Agriculture.
- ◆ National guidelines were subsequently passed by ministerial decree on September 2, 1997
- ◆ Field tests were approved in 1998 by the National Biosafety Committee for Bt cotton, Bt corn, Roundup Ready cotton, Roundup Ready corn, and Roundup Ready soybean. Multi-locational, unconfined trials have been conducted.

Impacts

Indonesia has been a major focus country for ABSP's capacity building in biosafety. Through workshops, internships, and consultants, Indonesian scientists and policy makers have been brought together to address regulatory issues relating to the testing and commercialization of transgenic crops. Along with Egypt, Indonesia has made significant progress in the development of biosafety guidelines and procedures.

◆ Biosafety Guidelines Developed and Approved

National biosafety guidelines were passed by ministerial decree on September 2, 1997, allowing Indonesian scientists and international companies and research institutions to field test transgenic crops in Indonesia.

◆ **Transgenic Crops Commercialized.**

Indonesia's Biosafety Committee has now given deregulated status to 5 transgenic crops (Bt cotton, Bt corn, Roundup Ready cotton, Roundup Ready corn, and Roundup Ready soybean from Monsanto) for unconfined multi-location trials. Later in 1999 they plan to conduct confined field trials of Bt corn from Pioneer Hi-Bred, and other crops are currently being tested in the greenhouse. The Ministry of Agriculture recently gave its approval to the limited sale of transgenic cotton. An application for a confined field trial of ABSP's potato tuber moth resistant Bt potato was submitted to the Indonesian Biosafety Committee and USAID's Biosafety Committee in 2000, and a field trial is planned as soon as approvals are granted.

◆ **GMO Food Safety Guidelines Developed.**

Supported by ABSP, Dr. Muhammed Herman and Dr. Achmad Hidayat, Central Research Institute for Food Crops (CRIFC) attended the International Food Safety course at Michigan State University (MSU) in 1999. Drs. Herman and Hidayat were subsequently appointed to the committee charged with drafting Food Safety Guidelines for GMOs in Indonesia. The Ministry of Agriculture and other relevant ministries in Indonesia have since approved these guidelines.

Indonesia IPR/Technology Transfer

Activities

◆ **Intellectual Property/Patent Internship Program, Stanford University, April 1993.**

An IPR internship program was designed and implemented by Professor John Barton of Stanford Law School from April 1-30, 1993. Seven interns from Egypt, Kenya and Indonesia participated in the program, the goal of which was to provide hands-on experience to legal and scientific personnel from developing countries in various issues related to intellectual property rights. In addition the internship enhanced communication between those involved in the sciences and those with responsibilities in the legal issues surrounding biotechnology. The program encouraged the assessment of current intellectual property structures within the participants' home countries, provided access to literature and expertise regarding IPR in both the public and private sectors.

◆ **IPR Workshop, Washington DC, July 1994.**

ABSP sponsored this workshop in Washington DC from July 11-14, 1994 as a follow up to the Egypt workshop. Forty-four participants attended from Egypt, Kenya, Indonesia and Costa Rica, Thailand, Sri Lanka as well as a number of institutions and agencies such as USAID and the World Bank. The purpose of the workshop was to present intellectual property rights in biotechnology as an important issue to institutions and individuals. Proceedings of this workshop were published: *Intellectual Property Rights, Proceedings from the ABSP Workshop Series July 11 - 14, 1994, Washington, D.C.*

◆ **Plant Variety Protection and Patents Workshop, Indonesia, 1996.**

ABSP, through the support of USAID/Jakarta organized a two-day workshop on intellectual property rights in agriculture from March 25-26, 1996, which was attended by

fifty senior representatives from the government and private sector in Indonesia. The workshop was organized in collaboration with the Central Institute for Food Crops (CRIFC) in the Indonesian Ministry of Agriculture. The main goal of the workshop was to assist Indonesia in drafting their new plant variety protection law.

Impacts

◆ **Establishment of Technology Transfer Office**

Through training and technical assistance from ABSP, the Agency for Agricultural Research and Development (AARD; equivalent to the Agricultural Research Service/USDA) established a new office of Intellectual Property and Technology Transfer in Bogor, Indonesia, July 1999. The ABSP office trained two staff members in various issues of IP management and technology transfer. The office (known by the Indonesian acronym KIAT) is now actively involved in educating scientists and policy makers in Indonesia in management of IP. KIAT is also working with the private sector to license technologies generated within AARD institution and will serve as the main focal point for management of intellectual properties related to agriculture/biotechnology. The AARD is one of the few developing country institutions to recognize the benefits of intellectual property and to develop within the ministry a system for protecting and exploiting Indonesian innovations to benefit Indonesian agriculture. Within 3 months of its operation, KIAT has executed 5 license agreements to commercialize a wide range of technologies developed by the AARD institutions.

According to Dr. Achmad Fagi, Secretary General of the AARD, this office is the direct result of training received in IPR and Technology Transfer at MSU via the short course. The office will have a legal and financial division, general business division, technical division, and a secretariat. Ketty Karyati, who has received training as part of ABSP's capacity building efforts with Indonesia, will be the administrator of the office as the secretary. KIAT has expressed interest in running an in-country IPR workshop to educate key scientists and various AARD institutions. In addition, MSU's draft IP policy was shared with KIAT to be used as a basis for developing a system-wide policy in IP.

Since it was established, KIAT has licensed 43 plant varieties, using trademark licensing and technologies such as biofertilizer and biopesticides. Most recently they have licensed two rice hybrids varieties. It is hoped that the office will be self sufficient in 2004.

◆ **Founding of the Indonesian Inventor Society.**

Dr. Didiek Hadjar from the Estate Crops Research Institute attended the MSU IPR and Technology Transfer Course in 1998. He has since co-founded a new organization called the Indonesian Inventor Society and is serving as President. Again, this organization was developed as a direct result of Dr. Didiek's participation in the course. There have been several biofertilizer/biofungicide technologies patented with the assistance of this organization and are in various stages of commercialization.

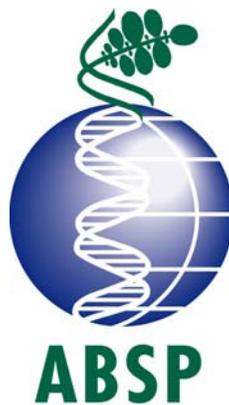
◆ **Indonesian PVP Law passed in 2001.**

In December 2000, the Indonesian Parliament approved the Plant Variety Protection (PVP) Act. This law is based on the UPOV 1991 Convention. ABSP assisted in drafting this new PVP law in 1995, and researchers trained by ABSP have been working with the Minister of Agriculture to educate the Parliament about the law.



Biotechnology Research and Policy Activities of ABSP In Costa Rica

1991--2002



The Agricultural Biotechnology Support Project

Supported by the United States Agency for International Development (USAID) and implemented by Michigan State University under Cooperative Agreement: DAN-A-00-91-00126-00

ABSP Biotechnology Research and Policy Activities in Costa Rica

1991-2001

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Micropropagation of tropical crops for commercial planting (Costa Rica)

DNA Plant Technology (DNAP), USA
Agribiotecnología de Costa Rica (ACR), Costa Rica

1991-1997

Project Goal

To implement more efficient methods of micropropagation of banana, pineapple, coffee, and ornamental palm for high-quality commercial planting stock. Banana, and pineapple are increasingly important export crops for Costa Rica and Central America, and tissue culture methods help to eliminate problems due to insects, nematodes and some fungal diseases. However, tissue culture is expensive and conventional techniques are not able meet the high demand for plants. The goal of this project was therefore to investigate the potential of embryo regeneration using bioreactors as an efficient, low cost way to increase production of pineapple and banana.

Project Activities

ABSP's initial private sector collaborator was DNA Plant Technology, a plant biotechnology company in Oakland, California, which came to the project with a host country partner in place-- ACR in Costa Rica, a micropropagation company with laboratory and farm facilities in Costa Rica and an export business in tropical crops and ornamentals. Later, DNAP also had an agreement with Fitotek Unggul in Indonesia (see next section).

The agreement between ACR and DNAP was initiated in 1992 with the general goal of implementing more efficient methods of micropropagation of banana, pineapple, coffee, and ornamental palm for high-quality commercial planting stock. ACR was to provide the germplasm of all the target crops. DNAP was to develop the micropropagation technologies, transfer to ACR the processes and material for field trials, and commercialize the final product. The agreement stipulated that technical training be provided for ACR staff. ABSP provided seed money to support the project.

Project Impacts

Although the project was disrupted by some ownership, location, and personnel changes at DNAP and a decrease in ABSP funding of the project midstream, this private-to-private linkage had a number of positive outcomes:

- ◆ ACR now has a practical pineapple micropropagation system and is following some promising lines in banana regeneration. The collaboration with the ABSP project improved ACR's efficiency in producing banana and pineapple planting materials.
- ◆ Preliminary results for somatic-embryogenesis-derived pineapple plants indicated a much higher than usual rate of variation in plant and fruit size, and other factors.
- ◆ The technology was not successful for banana due to problems with replicating published research and with bacterial contamination. Both DNAP and ACR have continued with banana research and have been optimistic about future success.

- ◆ DNAP provided technical training to ACR staff in the use of short-term bioreactor technology and transformation technology. Three senior scientists from DNAP spent time at SCR providing technical training. Both sides felt that this was very much a co-learning experience.
- ◆ ACR scientists, through the training component of the agreements, became important participants in their countries' biosafety and plant protection policy efforts. Professional liaison and mutual respect established among scientists at DNAP and ACR have resulted in future informal cooperative efforts.
- ◆ The techniques for coffee micropropagation was developed in the early stages of the project and with ABSP funding several hundred plants were later transferred to ACR in Costa Rica for testing. However, organizational changes at DNAP necessitated the discontinuation of this part of the project. However, ACR continued field-testing of plants from earlier work.
- ◆ A small exploratory project with palms was carried out, and of 4 species of palm, peach palm was found to be the most amenable to tissue culture techniques. After budget cuts in early 1994 DNAP work on palm was discontinued. DNAP provided ACR with a zygotic embryo propagation method for peach palm, but this proved to be unfeasible due to the lack of cross pollination in peach palm.
- ◆ Through ABSP's website we continue to pass enquiries on micropropagation and the availability of micropropagated crops to ACR.

This project was successful because DNAP and ACR had a common private-sector culture that included a level of trust and a similar understanding of contract law and confidentiality. The final project assessment report points out that the venture was also successful because it supported co-learning rather than one-way technology transfer. Staff at ACR report that the work they have carried out with ABSP's involvement has enhanced the reputation of their company.

Costa Rica Biosafety

Activities

- ◆ **Latin America & Caribbean Region Biosafety Workshop, Jamaica, May 1993.**
ABSP organized a regional workshop in Jamaica, in collaboration with the Bean/Cowpea CRSP in May 1993. 42 representatives from 12 countries attended the workshop the objectives of which were: i) to examine the status of biosafety guidelines and regulations in the region for testing and utilization of genetically engineered food crops; and ii) to assist participants in developing work plans and recommendations from which to begin building the necessary biosafety policies and guidelines in their own countries. Proceedings of the workshop were published. [*Proceedings of the USAID Latin America Caribbean Region Biosafety Workshop, May 10 - 13, 1993, Oracabessa, Jamaica.*]

Costa Rica IPR/Technology Transfer

Activities

- ◆ **IPR Workshop, Washington DC, July 1994.**
ABSP sponsored this workshop in Washington DC from July 11-14, 1994 as a follow up to the Egypt workshop. Forty-four participants attended from Egypt, Kenya, Indonesia and Costa Rica, Thailand, Sri Lanka as well as a number of institutions and agencies such as USAID and the World Bank. The purpose of the workshop was to present intellectual property rights in biotechnology as an important issue to institutions and individuals. Proceedings of this workshop were published: *Intellectual Property Rights, Proceedings from the ABSP Workshop Series July 11 - 14, 1994, Washington, D.C.*
- ◆ **IPR and Technology Transfer Internship Program at MSU, 1996.**
A two-week internship program in IPR and technology transfer was organized at MSU from February 4-17, 1996. This was organized by ABSP in cooperation with the Office of Intellectual Property and the Institute of International Agriculture at MSU. The goal of the program was to provide hands-on experience to international scientists, administrators and policy makers in the day to day handling of intellectual properties within the context of recent changes in the GATT agreement. To foster networking, the participants also attended the annual meeting of the Association of University Technology Managers (AUTM) in South Carolina.

The success of ABSP's first internship program led MSU to develop it into a short course that has now been offered annually since 1996. The course is run in collaboration with the Institute of International Agriculture and the Office of Intellectual Property. The focus of this one-week program is on IPR and technology transfer education with special emphasis on day-to-day handling and management of intellectual properties as it relates to agriculture. We believe that this is still the only structured short course held in the US that covers the IPR and technology transfer issues related to agriculture.

During the last five years, 84 international participants have attended this program. The ABSP project has directly sponsored participants from the following countries: Costa Rica

(1), Egypt (4), Kenya (2), Morocco (5), Indonesia (6), South Africa (1), India (2) and Ethiopia (1).

◆ **East Africa IPR Workshop, Uganda, 1999.**

ABSP held a workshop on *The Impact of Intellectual Property Rights on International Trade and Agriculture in East Africa* in Kampala, Uganda from January 18-20, 1999. The Ugandan Council for Science and Technology (UNCST) assisted ABSP in the local organization of the workshop. Additional funds for the support of regional participants to attend the meeting were obtained from the Technical Center for Agricultural and Rural Cooperation (CTA, Netherlands), the Rockefeller foundation and Monsanto. Over 70 participants attended the workshop from Ethiopia, Kenya, Nigeria, Tanzania, Uganda, Zambia, Zimbabwe, Switzerland, United Kingdom, France, United States, Costa Rica, South Africa, and the Netherlands.

◆ **Linkages with the Association of University Technology Managers (AUTM), 1995-2001.**

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 Indonesia (7), Costa Rica (1), South Africa (1), Egypt (4), Morocco (5), and Kenya (2) to attend the annual or regional meetings of AUTM in the US.

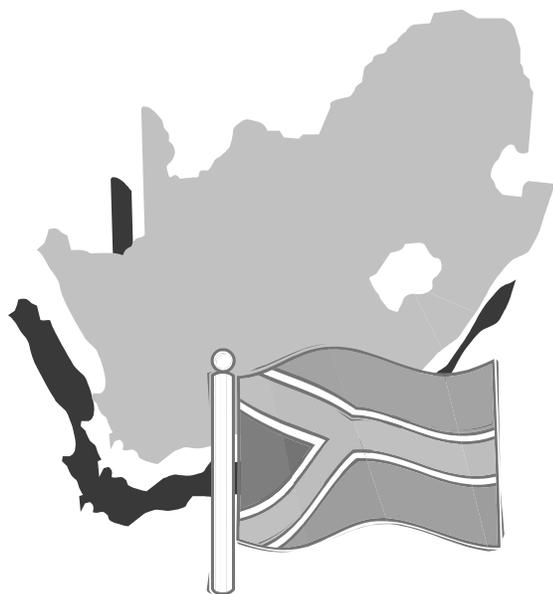
◆ **ABSP Industrial Seminar Series**

In April 1993, the ABSP organized an Industrial Seminar Series (ISS). The ISS was organized to provide opportunities for senior scientists and administrators from the public and private sector, and government officials from the ABSP partner countries (Costa Rica, Egypt, Indonesia, Kenya) to interact with technical and business personnel at private biotechnology companies in the U.S. that have active agricultural biotechnology programs. In addition to seven participants from the ABSP partner countries, three participants from Jamaica (sponsored by the USAID-Jamaica) also attended the ISS. The companies visited included Garst/ICI Seeds, Inc. (now Syngenta), Ecogen Inc (now part of Monsanto), and DNA Plant Technology.

The ISS was instrumental in opening lines of communication between developing country leaders and host companies. It also provided participants an exposure to a diverse group of companies oriented towards different end-user groups.

Other impacts of the ISS included:

- ◆ A Memorandum of Understanding (MOU) was developed between Agriobiotechnologia de Costa Rica and Fitotek Unggul (Indonesia) to collaborate in tissue culture and micropropagation of bananas and other horticultural crops.



Biotechnology Research and Policy Activities of ABSP in South Africa

1999--2003



The Agricultural Biotechnology Support Project

Supported by the United States Agency for International Development (USAID), implemented by Michigan State University under Cooperative Agreement: DAN-A-00-91-00126-00

ABSP Biotechnology Research and Policy Activities in South Africa

1991-2003

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Bt Potato Field testing in South Africa

*ARC- Roodeplaas Vegetable and Ornamental Plant Institute, Pretoria,
South Africa*

1999-2003

Project Goal

Potato (*Solanum tuberosum* L.) is an important vegetable crop in South Africa. The majority of South Africa's production lies in four provinces: Mpumalanga, the Northern Province, Eastern Free State, and the Western Free State. There are 14 different potato-producing regions in South Africa, occupying diverse climates. In some of these regions, potatoes are produced year round. One of the major potato production constraints is the potato tuber moth (PTM). Damage caused by PTM reduces the quality of tubers, with resulting reduction in marketable yield. The South African potato crop moves through several different channels. Total potato production is split into table potatoes and seed potatoes. The food sector of the potato industry is comprised of two distribution channels: the fresh market sector and the 'not-on-market' sector. Aside from the formal channels, in rural areas potatoes are sold in local markets or informally. In 2000, only 3% of the total potato production was exported, mainly to neighboring countries. The overall objective of the project is to field test transgenic potatoes with resistance to potato tuber moth at various locations in South Africa.

Project Activities

- ◆ Obtain a permit for importation of a GMO product as well as a permit for importation of new potato material
- ◆ Tissue culture multiplication and Mini-tuber production of Spunta G2 and G3 material to bulk up for Field trials
- ◆ Biosafety applications to the GMO authorities for field trials in 2 locations in South Africa
- ◆ On station field trials at Roodeplaas (VOPI) for 2 years and a on-farm field trial at Ceres, Western Cape for 1 year
- ◆ Storage trials at VOPI for 2 years

Project Impacts

- ◆ The MSU-developed PTM resistant lines of the cultivar Spunta lines were field-tested by VOPI in 2001/02 at Roodeplaas, Pretoria. In the first year of trials the Spunta-G2 and Spunta-G3 lines were free of tuber moth damage (foliar and tuber) in the field, and highly resistant to the tuber moth in diffused light storage tests.
- ◆ During the second year of trials (2002/03), field tests were carried out at Roodeplaas as well as in Ceres (Western Cape province), a major table potato and seed-potato producing region of South Africa. At Roodeplaas, no tuber moth damage was observed in the foliage despite the release of 30,000 moths. Under natural infestations at Ceres, Spunta-G2 and Spunta-G3 were free from potato tuber moth damage in both foliage and tubers. Tuber yields at Ceres were high, with Spunta-G2 and Spunta-G3 showing similar yields. The yield of non-transgenic Spunta and the two transgenic lines was 30% higher than BP1, a major South African cultivar, because no PTM tuber damage was recorded.

However, in post-harvest storage, PTM infestations will occur, and tuber losses are reported up to 100% in the non-transgenic cultivar.

- ◆ Storage tests comparing Spunta-G2 and Spunta-G3 with their non-transgenic counterpart showed 100% protection from PTM, while all non-transgenic Spunta became infested.

Southern Africa Regional Biosafety (SARB) Program: 2000 - 2003

Overall Project Goal

- The overall objective of this program was to build regional policy and technical capacity to support science-based regulation of the development, commercial application, and trade in agricultural products derived from modern biotechnology in the Southern African region.
- The specific objective was to lay the regulatory foundation that will support the field testing of genetically engineered products (e.g. crops or livestock vaccines) in four of the seven target countries in the SADC region by 2003.
- The program focused on seven SADC countries: Zambia, Zimbabwe, Mozambique, Mauritius, Namibia, South Africa and Malawi. The Vegetable and Ornamental Plant Institute (VOPI) of the Agricultural Research Council in South Africa was the lead contractor on the program. Golden Genomics (previously known as Innovation Biotechnology), a private consulting firm, was also involved in the development of the program as a sub-contract to VOPI.

Activities

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

Activity 2: Regional Workshop on Biosafety: This workshop served as a general awareness-raising event on biosafety in the SADC region. It was targeted towards legislators/policy makers, regulators, members of biosafety committees as well as delegates to the Cartagena Protocol on Biosafety and Codex.

Activity 3: Regional Biosafety Training Course: The purpose of this training course was to train regulators and reviewers (preferably not previously trained) in biotechnology and biosafety issues.

Activity 4: Journalists/Media Course: Media reporting on biotechnology is increasingly influencing how policy makers develop and implement biosafety regulations as well as public perceptions regarding biotechnology. The purpose of this workshop was to provide balanced information on biotechnology and biosafety to key media in target countries. It was also to address issues of how policy makers/regulators convey issues of safety and regulation to the media.

Activity 5: National Follow-up/In-country Biosafety Training: Pending funding levels and progress by target countries in discussion of regional policy cooperation, national-level biosafety training will be held as a subset of the target countries. The purpose of these national biosafety training activities will be to broaden the range of policy makers with biosafety training to include all members of National Biosafety Committees and other stakeholders such as Ministries of trade, industry, farmers organizations, etc. National Biosafety training courses will be presented in the 7 Core Target countries. These courses will vary to meet the specific needs of each country.

Activity 6: Risk Assessment Research-Sorghum Gene Flow Case Study: A critical component of biosafety risk assessment and management will be knowledge about

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

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

Project Achievements

1. More than 600 people sensitised to biosafety within the region.
2. 380 scientists trained in the basics of conducting a risk assessment of GM crops or products.
3. A regional network established through which biosafety issues can be promoted and deliberated.
4. Three countries now have legislation on GMOs while another three have draft legislation due to be presented to their respective parliaments shortly. The last country, Mozambique, has instituted regulations for dealing with GM food aid and has begun the process of drafting legislation. SARB has contributed towards all these processes and improving the implementation of regulations in those countries which already had legislation.
5. SARB contributed greatly to the heightened awareness of GMO technologies and the safety thereof within the region. This was mainly through its training of journalists and conducting workshops for policy makers and public.
6. The greatest success lay in the in-country training whereby each country through its Working Group members, established its own training needs, developed its own work plan and assisted SARB in executing it.

South Africa: SARB In-Country Training

Activities

This activity was designed to meet the priority biosafety training needs in South Africa.

The South African working group under SARB identified the need for three training workshops. These were held sequentially in Pretoria. The first course was a three-day scientific training on biosafety for members of the Scientific Advisory Committee and scientific reviewers attached to this body. These 28 delegates were mostly experienced in risk assessment, but the ABSP Biosafety workbook was supplied as a reference document and referred to in the case studies and for homework. A specific case study was developed at the request of the working group. This generated considerable discussion. The confidence and experience of this group was evident in the level and intensity of discussion. The course closed with a general discussion on the needs of the group and their requirements for further capacity building. The group agreed to assist with regional biosafety development as and when they could. The Registrar of the GMO Act attended the course and made a commitment to follow-up the workshop with similar meetings to enable discussion and training for these scientists.

The second workshop was held for the Executive Council, the national GMO decision making body in the country. This was the first time this group was given biosafety training and the course was a condensed introduction to the elements of risk assessment and biosafety implementation. These courses were presented by Dr Donald Mackenzie (Canada), Dr Graham Thompson, Mr Gurling Bothma and Mrs Muffy Koch. There was considerable discussion, much of which was clarity sought from the group and discussion on processes and mechanisms needed for smooth implementation. They committed themselves to introduce a fast tracking system for applications that meet certain requirements and to participate in regional biosafety activities.

The third course was a two-day training for government inspectors from the Department of Agriculture. There were 21 delegates from all provinces and the course was designed to build capacity and develop systems for these regulators. The level of experience in the senior inspectors was impressive and these delegates facilitated the workgroups. They will be valuable course facilitators for further training in SADC countries. The discussion was lively and led to consensus on a number of process issues and development of guidelines for GM inspections.

Ms Michelle Vosges, two of the local inspectors, Mrs Koch and Mr Bothma presented this course. Ms Vosges carried the major load in organising the workshops and put considerable effort into this activity.

Impacts/Outcomes

These courses were the first capacity building exercises in South Africa since the implementation of the GMO Act in December 1999. While well overdue, the courses set sound biosafety principles and sensitised the officials to the need for annual training and interaction between biosafety regulatory groups. This was deemed necessary to ensure ongoing capacity development and process review in order to enable effective biosafety implementation. As such, the SARB training initiated a process of ongoing capacity building that is seen to be important to the delivery of an effective biosafety service.

ABSP Biosafety Workbook

ABSP developed a manual for biosafety training: **Biosafety & Risk Assessment in Agricultural Biotechnology: A Workbook for Technical Training**. Patricia L. Traynor, Robert J. Frederick, Muffy Koch, Published by The Agricultural Biotechnology Support Project (ABSP), Michigan State University. (ISBN: 1-56525-016-8). Designed to complement technical biosafety-assessment training courses in developing countries, this workbook provides a background for the practical application of biosafety review procedures using a case study approach. The intended audience includes members of national biosafety committees, biotechnology regulatory officials, and scientists working in the public and private sectors. It is a useful resource for national decision-making bodies, government regulators in related areas, and those charged with monitoring approved field-test releases. The workbook is the product of biosafety experts with years of experience in technical as well as information-oriented training. It is organized in three parts.

The workbook was successfully used at the SARB regional training course in November 2001 and during subsequent in-country training courses presented in SARB target countries. Although initially designed to accompany training workshops conducted under SARB; it has been quickly taken up and used in training events sponsored by other capacity building programs, including those of USDA and ISNAR, and will be used in the newly implemented USAID Program for Biosafety Systems. Since publication in January 2003, approximately 250 print copies have been distributed to individuals world-wide; and another 350 used in training courses. In addition, it is freely available for download from http://www.iaa.msu.edu/absp/biosafety_workbook.html, and this web page has been accessed over 2,000 times since January 2003. Currently the workbook is being translated into French, Portuguese, and Spanish for use in those world regions. Translation into additional languages, including Indonesian is under consideration. Although there are no plans to print these versions, they will also be available to download from the ABSP Website. The workbook's authors are also in the process of developing a companion "teachers" volume, which will be available in electronic form.

Budget Information