Agricultural Biotechnology Support Project II

A B S P

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INTRODUCTION

The extent to which genetically modified (GM) crop technologies can catalyze global development, especially as they benefit poor and hungry people, depends on how effectively the global scientific communities, including both the public and private sectors, safely harness a broader choice of GM crops for a wider range of developing countries. Over the past 12 years, Cornell University has addressed this issue by leading the Agricultural Biotechnology Support Project II (ABSPII; http://absp2.cornell.edu), a pioneering project comprised of an extensive consortium of public and private partner institutions. The goal of the project was to develop new GM crop varieties for which there was a demand from developing countries, especially in cases where conventional plant breeding methodologies or agronomic innovations were not feasible.

The ABSPII initiative filled an important gap. The large multinational companies that have been the main drivers of global GM crop commercialization do not focus on the crops and trait combinations addressed in the ABSPII project. Such companies typically invest in the constraints that affect major crops that have high commercial value and extensive international markets. The ABSPII project has instead focused on developing and commercializing GM crop varieties of the minor or orphan crops that have the potential to improve the lives of millions of people in the developing world, particularly small-scale farmers. Priority setting studies in Africa and Asia helped ABSPII narrow down the list of specific crops and traits to focus on for future product development efforts that would lead to commercialization.

ABSPII was a remarkably successful project. It is to date the only project funded by USAID that has developed and commercialized an important GM food crop by a Feed the Future developing country. Significant outputs from ABSPII in developing countries included: (i) improved capacity for research, product development, licensing and communication and marketing through hands-on training; (ii) enhanced access to technology and intellectual properties; and (iii) the development of several new GM crops. Potential outputs of ABSPII include: (i) delivery of high quality seeds to resource-poor farmers; (ii) major improvements in crop yields; (iii) lower production costs; (iv) reduced pesticide use resulting in less pesticide residue in the environment; and (v) improved human health.

This final report highlights how ABSPII has achieved these successes. It also addresses challenges that have been and, in some cases, are still encountered by the project, especially in the face of politically adept anti-GM crop zealots whose arguments are often based on emotion and pseudo-science rather than scientific facts. Finally, we discuss lessons learned that could inform future initiatives with similar goals.

ABSPII BACKGROUND

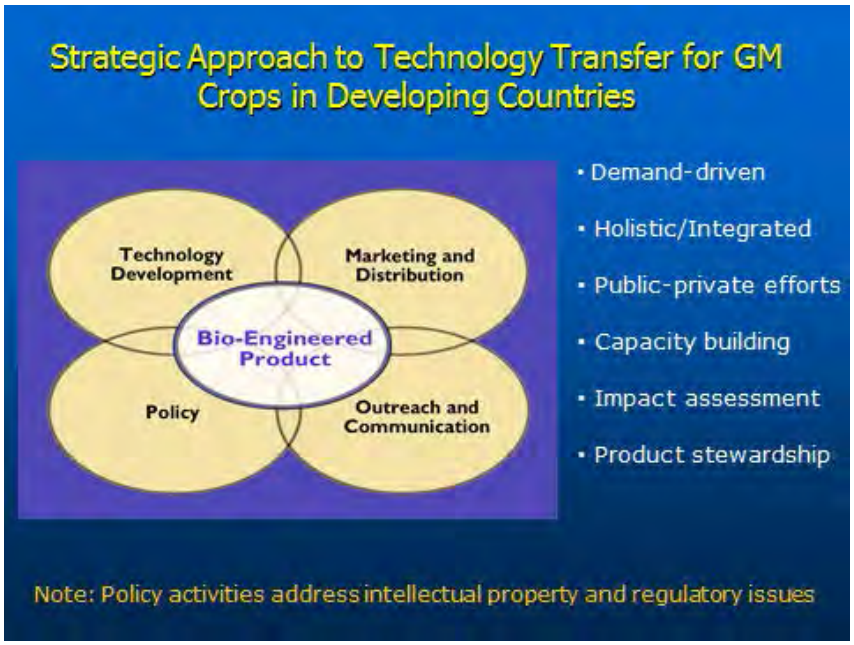
Cornell University was awarded the funding for ABSPII because of its innovative and new strategic approach to building capacity and delivering products in the area of genetically modified crops. Over the past 12 years, implementation of the ABSPII concept has led to major advances in research, development, capacity building, awareness and commercialization as it relates to new GM crop varieties for South Asia, Southeast Asia, East Africa and West Africa. All ABSPII activities were demand-driven with a holistic and integrated approach that considered every relevant technical and non-technical issue from the outset. The ABSPII strategy was discussed in detail by Gregory *et al* (2008) (Bioengineered Crops as Tools for International Development: Opportunities and Strategic Considerations. Experimental Agriculture, volume 44, pp. 277-299). The following provides some highlights of that strategy.

Through priority setting exercises that included discussions with public and private sector partners, the first step in the strategy was to determine whether there was a strong local demand for a potential new GM crop. Only crop/trait combinations that could not be addressed using conventional technologies such as breeding or effective IPM strategies were selected for consideration. The next task was to assess the feasibility of developing and delivering the potential products following SWOT (strengths, weaknesses, opportunities, and threats) analyses. Prioritization criteria included: 1) access to existing or experimental technologies/intellectual properties that would provide a high probability of success; 2) a socio-economic benefit that would justify the product development costs; and 3) availability of public-private partners capable of developing the chosen crop/trait combinations in correct germplasm.

Once the initial portfolio of potential new GM crops was finalized, we were highly proactive in establishing linkages among the various partners needed to move the crop from the biotechnology research and development stage to delivering seeds into the hands of farmers. Building partnerships with the public and private sectors comprised the strategic and operational foundation of ABSPII. Depending on project specifics, partners included universities, national and regional research organizations, private multinational and local companies, non-governmental organizations, government agencies, international agricultural research centers, and other stakeholders from developing and industrialized countries who are involved in the research-development-commercialization-delivery continuum.

With strong partner participation, we then organized the research, development and commercialization work for each new GM crop product into individual Product

Commercialization Packages (PCPs). The main components that comprise each PCP are illustrated in the figure below.



Source: Gregory *et al.* (2008)

Each PCP integrated all elements of the research, development and commercialization processes. These included: (i) technology development; (ii) policy-related issues such as licensing the intellectual properties associated with the product as well as preparing for, and applying for, regulatory approval by the relevant national authorities; (iii) providing public information to producers and consumers about the benefits, risks and correct management of these new products; and (iv) establishing, or verifying, the existence of marketing and distribution mechanisms to provide farmers access to planting materials.

Product-focused, "hands-on" capacity building, impact assessment, and stewardship were also built into all activities of ABSPII. Our product development and commercialization strategy also included a decision-making process for terminating products that were unlikely to fit our mandate of introducing GM crops into countries in the foreseeable future.

ABSPII's progress in technology development was prolific and cost effective. Our approach of developing a popular cultivar containing a trait from a single event and building traits into various additional cultivars through backcross breeding provided opportunities for public enterprises to develop several regionally relevant varieties without incurring significant capital cost. For example, in South Asia, 20 varieties of eggplant, four varieties of potatoes, and one variety of rice were chosen for incorporating relevant traits through strong public and private

sector partnerships. ABSPII cumulatively invested only $3.7 million in the region, but buy-in investment of $2.5 million from various public and private partners operating in the target countries significantly helped to promote rapid progress while reflecting national enthusiasm for the ABSPII approach.

Capacity building was one of the most important key elements of ABSPII. Our objective was to support national research institutions by helping them invest in infrastructure and equipment and provide advanced technical and product management training and provide degreed educational opportunities. ABSPII used a "product-driven capacity building" approach to help strengthen developing countries' research, regulatory, and distribution systems. The idea was to work through applicable technologies that local extension workers and farmers were demanding to help with specific and persistent problems. This pressure makes it imperative for local researchers and policy makers to work through the research and validation phases, follow through on each step of the regulatory approval process and consider the real-world implications of making the technology available to farmers.

In addition, we provided opportunities to improve our partners' institutional capacity to manage large grants and execute licensing and intellectual property agreements. We hosted numerous workshops to provide information and training in the areas of socio-economics associated with the adoption of GM crops, the use and generation of intellectual property, risk management and biosafety, product development, communications and commercialization activities including seed scale-up and distribution. Hundreds of farmers, researchers, policy makers and professional communicators were educated over the 12 years since the inception of ABSPII. Such product-driven capacity building in developing countries may be one of ABSPII most important legacies.

In the planning and implementation of the PCPs, care was taken not to underestimate the resources needed to move a GM crop from the research phase into the hands of the end-user; a point often overlooked by public sector organizations such as universities and national research institutions which, historically, focus almost exclusively on research. Costs associated with product development and commercialization typically constitute two-thirds of total investment: included in these phases are the regulatory, seed multiplication, marketing and distribution.

Obtaining regulatory approval for new GM crops can be a particularly costly bottleneck for commercialization of any GM product. ABSPII compiled a regulatory package associated with commercialization of each GM crop product. Compiling such a package from scratch can cost millions of dollars and involve up to a decade of research. Thus, to the extent possible, ABSPII

utilized information from existing regulatory dossiers generated in other countries for crops with similar or identical transgenes.

Depending on the country involved, regulation is governed by national biosafety legislation and the authorities responsible for its implementation. As much as possible, ABSPII compiled regulatory dossiers in compliance with the sovereign laws dictating the regulatory process in the target countries where we worked. Much of what is needed in a regulatory dossier is codified; however, some of the work involved negotiation and addressing widely different perceptions of risk. Country-specific data for the regulatory packages were generated as much as possible by ABSPII and its partners within the focus country or region. Whenever possible, ABSPII and our partners solicited professional advice from competent regulatory consultants possessing industrial experience deregulating GM crops in other countries. An interactive relationship with regulatory authorities was established from the outset of product development and dialogue was maintained throughout the time leading up to the formal submission of the regulatory packages.

INDIVIDUAL PRODUCTS

Over the past 12 years, ABSPII was engaged in developing GM products for partner countries: India (fruit and shoot borer resistant eggplant, and late blight resistant potato), Bangladesh (fruit and shoot borer resistant eggplant, and late blight resistant potato), Indonesia (late blight resistant potato), the Philippines (fruit and shoot borer resistant eggplant) and Uganda (disease and nematode resistant East African Highland Banana). Products originally supported by ABSPII which reached various stages of GM product development, but were eventually discontinued for various reasons, included virus resistant tomato for West Africa, virus resistant cassava for Kenya, pod borer resistant chickpea and virus resistant ground nut for India, drought tolerant rice for Bangladesh, and papaya ringspot virus resistant papaya for the Philippines.

A brief description of the participating countries, partnering institutions, technology used, and current status of the product is shown in tabular form for each of ABSPII's GM crop products. Products discontinued during the first phase of ABSPII will be described, but discussed in less detail. The work that needs to be done to complete commercial release and the lessons learned are described below the tables.

Fruit and Shoot Borer Resistant Eggplant: India, Bangladesh and the Philippines

Product and Participating Countries	Partnering Institutions	Technology	Current Status
Fruit and Shoot Borer Resistant Eggplant Bangladesh, India, Philippines	• Bangladesh Agricultural Research Council • Bangladesh Agricultural Research Institute • Government of India, Department of Biotechnology • Indian Institute of Vegetable Research • International Service for the Acquisition of Agribiotech Applications, Philippines (ISAAA) • Lal Teer Seeds Ltd., Bangladesh • Maharashtra Hybrid Seed Company, India • Sathguru Management Consultants, India • Tamil Nadu Agricultural University, India • University of Agricultural Sciences, Dharwad, India • Department of Agriculture, Philippines • University of the Philippines Los Baños • University of the Philippines Los Baños Foundation • University of Southern Mindanao, Philippines • Central Bicol State University of Agriculture, Philippines Southeast Asian Regional Center for Graduate Study and Research in Agriculture, Philippines • Virginia Polytechnic Institute and State University, USA	• *cry1Ac* is a gene from the naturally occurring bacterium, *Bacillus thuringiensis* (Bt) • Bt produces an insecticidal protein that has a toxic effect on many caterpillars, including the eggplant fruit and shoot borer. • This is a major insect pest of eggplant in many parts of the world and is very difficult to control using conventional methods. • Bt crops like Bt cotton, expressing the same Cry1c protein in Bt eggplant have been commercialized worldwide • Transgenic eggplants containing the Mahyco event 'EE-1' express the Cry1Ac protein have been developed that effectively control the eggplant fruit and shoot borer. • The Bt eggplant event technology was acquired pro-bono from Mahyco in India.	• Partners in India developed transgenic Bt eggplant varieties • Bt eggplant event EE-1 was introgressed into locally desirable eggplant varieties for India, Bangladesh and the Philippines. • In 2009, Bt eggplant environmental and food safety for commercial release was approved by India's Genetic Engineering Approval Committee (GEAC) • Commercialization of the crop in India is under a moratorium imposed by the Ministry of Environment and Forests on Feb. 9, 2010 and yet to be released for commercial propagation. • In Bangladesh, four Bt eggplant varieties were deregulated and commercialized on 30th October 2013. • Under limited release, 20 farmers received Bt eggplant seedlings from the Minister of Agriculture in Bangladesh on 22nd January 2014 during the first season. In the second eggplant growing season, 108 farmers received Bt eggplant seedlings from BARI for cultivation. • Ex-post socio-economic study has been initiated in 19 districts of Bangladesh to gauge the benefits to farmers through the adoption of Bt eggplant. • BARI is working towards seeking regulatory approval for the release of remaining 5 Bt eggplant varieties. • Partner in Bangladesh (BADC) will carry out large-scale seed multiplication for seed dissemination to larger set of Bt eggplant farmers. • Partners in the Philippines developed 7 Bt eggplant varieties and hybrids for the Philippine market. Multi-location field trials in major growing areas for environmental risk assessment and variety accreditation have been completed. A certificate of satisfactory completion has been issued. This is a key requirement for application for commercial cultivation in the country. • The regulatory dossier for commercial propagation in the Philippines is being prepared. Application for propagation has been delayed pending the resolution of the petition filed in the Philippine Supreme Court. • In addition to the Bt hybrids, two non-Bt hybrid near-isolines were approved for release by the National Seed Industry Council of the Philippines in 2013 These are the first eggplant

			hybrids developed and released by a public sector institution in the country. Two more hybrids are expected to be approved in 2015. • Ex-ante socio-economic analyses and have been conducted to assess potential benefits to producers and consumers; surveys have been conducted to assess public acceptance of Bt eggplants; pesticide residue studies have been conducted in the largest eggplant producing area to assess impact on health and environment

Eggplant (*Solanum melongena*), known in South Asia and Southeast Asia as brinjal or talong, respectively, is a widely cultivated vegetable in India, Bangladesh, the Philippines and elsewhere in Asia. It is the second most important vegetable in India and Bangladesh behind potato and the most affordable, so it is consumed in a wide variety of dishes popular throughout those countries. Eggplant is highly nutritious and culturally important to the people from India and Bangladesh who annually consume between 8 and 9 million metric tons produced on more than 500,000 hectares. In the Philippines, eggplant is the number one vegetable based on hectares planted. Eggplant is attacked by a diverse complex of insect pests and diseases, but the most serious insect pest is the fruit and shoot borer (FSB), *Leucinodes orbonalis*. FSB infestation commonly causes yield losses of 60 to 70 percent even after repeated insecticidal sprays. This excessive use of pesticides (20-100 applications per season) is a considerable hazard to human health and the environment. For decades, scientists have worked to develop a sound integrated pest management (IPM) approach to control FSB but to no avail. There is no genetic source of FSB resistance that can be bred into commercially available eggplant varieties using traditional methods.

Cry1Ac is a gene from the naturally occurring bacterium, *Bacillus thuringiensis* (Bt), that codes for a protein that is insecticidal to many caterpillars, including the eggplant fruit and shoot borer. Before ABSPII was launched, the Maharashtra Hybrid Seeds Company (Mahyco) had developed a fruit shoot borer resistant (FSBR) eggplant event "EE-1" genetically engineered to express Cry1Ac-like protein. Recognizing an opportunity to serve resource poor, small-scale farmers, ABSPII's partner, Sathguru Management Consultants Pvt. Ltd. based in India, facilitated an arrangement by which Mahyco donated this technology to public sector partners in India, Bangladesh and the Philippines. Mahyco sublicensed the Cry1Ac technology on a royalty-free basis to the public sector partner institutions so the technology was made available to our partners on a royalty free/cost of recovery arrangement. As a result of this donation and capacity building support by ABSPII, the public sector partners developed locally popular open pollinated varieties of transgenic FSBR eggplant. The varieties selected for introgression were those preferred by resource-poor, small-scale farmers in India, Bangladesh and the Philippines.

Meanwhile, Mahyco developed fruit and shoot borer resistant transgenic eggplant hybrids for broader commercial release and profit taking via technology fees. A specific product stewardship and monitoring strategy to be implemented by the licensees (our partners) to safeguard the interest of the licensors was also devised during the sublicensing process. Data from ex-ante socio-economic analysis suggested that resource poor farmers who adopt public sector Bt eggplant technology would improve their income sufficient enough to eventually become purchasers of the more desirable Bt hybrid eggplant from Mahyco. Thus Mahyco expected an increase in market share over time rather than experiencing market share erosion due to availability of inexpensive open pollinated seed from the public sector. Mahyco proved to be a great partner/technology provider that willingly provided valuable technical and financial support for all the partner product development and regulatory activities.

The scientists from partner institutes for the ABSPII project in India (Tamil Nadu Agricultural University, Indian Institute of Vegetable Research and the University of Agricultural Sciences, Dharwad), the Bangladesh Agricultural Research Institute (BARI, Gazipur) and the University of the Philippines Los Baños (UPLB) were trained on product development, molecular characterization and product efficacy validation by experts from Mahyco. Scientists from BARI were also trained on backcross breeding and selection of homozygous lines of transgenic eggplant. The seed delivery personnel were trained in COTS package that was developed specifically for the ABSPII project for inventory management of transgenic seeds. Scientists from the ABSPII partner institutions were also trained on regulatory aspects of transgenic products and commercialization with a focus on biosafety evaluation, Environmental Risk Assessment studies and preparation of a robust regulatory dossier. The scientists from partner institutes in India, Bangladesh and the Philippines were also trained on Intellectual Property protection and science communication strategies for GM crops.

The regulatory file development of Bt eggplant benefited tremendously from the expertise and experience of Mahyco, the first Indian company to commercialize Bt cotton hybrids in India. In addition to allowing ABSPII and partners access to data for the *Cry1Ac* gene construct used in both Bt cotton and Bt eggplant, Mahyco helped the partners streamline the development of a comprehensive environmental and food safety regulatory package for Bt eggplant event EE-1 for India and Bangladesh. The regulatory file compiled by Mahyco for event EE-1 for scientific review and deregulation in India was made available in the Ministry of Environment website (http://www.envfor.nic.in/divisions/csurv/geac/bt_brinjal.html). Frequent interactions with professionals from Mahyco and the Indian regulatory bodies in all three countries helped accelerate the regulatory process.

A proactive information, communication, and education strategy was also developed very early in project implementation to address anticipated public and market perception issues. The communication design followed the product development pathway so that the communication and outreach activities and messages were focused on the needs of specific stakeholders as the product moved from the development stage toward regulatory clearance, pre-commercial stage, and, eventually, the commercial stage. An important feature of our communication strategy was a regular survey of perception, attitude, and level of knowledge and information needs of stakeholders about biotechnology in general and Bt eggplant in particular. Our communication campaign was particularly successful in educating scientists, policy makers and others involved with the regulatory approval process. But, we underestimated the impact that the grassroots farmer/consumer misinformation campaigns of Greenpeace and other anti-GM-crop NGOs would have in undermining the commercial approval of Bt eggplant in India.

Current Project Status by Country and Continued Efforts Required for Successful Product Adoption

India: On 14 October 2009, the Genetic Engineering Approval Committee (GEAC) of India approved deregulation of transgenic Bt eggplant. This was a significant decision on the long road to becoming the first genetically engineered food crop in India. The Genetic Engineering Appraisal Committee (GEAC) is the apex body constituted in the Ministry of Environment and Forests under the "Rules for Manufacture, Use, Import, Export and Storage of Hazardous Microorganisms/Genetically Engineered Organisms or Cells 1989" in the Environment Protection Act, 1986. (http://www.moef.nic.in/major-initiatives/geac-clearances). Thus, the Minister of Environment and Forests, Mr. Jairam Ramesh, considered himself the final gatekeeper of genetically engineered crops in India. Unfortunately, Mr. Ramesh fell victim to the misinformation and pseudo-science campaigns administered by Greenpeace and other anti-biotechnology NGOs. The NGOs sent thousands of faxes and emails to Mr. Ramesh's office urging him to ban the cultivation of Bt eggplant soon after the GEAC's announcement to deregulate and release Bt eggplant for propagation. His country-wide open forum for public discourse was a Greenpeace-fueled fiasco for ABSPII. On February 9, 2010, Mr. Ramesh decided to impose a moratorium on the product until "such times independent scientific studies establish, to the satisfaction of both the public and professionals, the safety of the product from the point of view of its long-term impact on human health and environment" (http://moef.nic.in/downloads/public-information/Annex_BT.pdf).

A thorough study of Mr. Ramesh's report on the subject clearly suggested that the Minister was heavily influenced by political pressure from those opposed to biotechnology rather than by critical scientific and balanced judgments of the technology. Six of the National Academies of

Sciences in India reviewed the regulatory dossier and several government imposed reviews of the scientific evaluation of the product and confirmed the safety and socio-economic benefits afforded by the Bt eggplant technology. Despite these positive assessments of the scientific rigor of the technology, it is still unclear what the future of commercialization of Bt eggplant will be in India. For now, the moratorium on eggplant in India has been a victory for Greenpeace and a setback for all Indian agriculture. Thus, Bt eggplant continues to be caught in a political quagmire in India. In the meantime, other agricultural Indian scientists and agencies have been attempting to reverse the Minister's decision and reinitiate the testing of genetically engineered crops in India.

Bangladesh: The Government of Bangladesh deregulated and commercialized four different Bt eggplant varieties with event EE-1 from Mahyco in October 2013. Under limited release, 20 farmers received seedlings of these varieties from the Minister of Agriculture in January 2014. The first season was met with successful propagation of the Bt eggplant, however, it became clear that two of the four varieties were more susceptible to bacterial wilt disease than expected. All farmers witnessed excellent control of FSB infestation, but farmers with farms located in regions prone to water logging experienced early maturation of the crop. The second eggplant growing season was planted during the dry season. The Bangladesh Agricultural Research Institute (BARI) presented 108 farmers with two of the four varieties of Bt eggplant seedlings for cultivation in observation plots located throughout Bangladesh. Bacterial wilt was a problem in only 8 percent of the observation plots and all cases were attributed to flood irrigation practices that were highly discouraged by the on-farm research division (OFRD). As of mid-April 2015, across the 108 observation plots, no farmers experienced more that 0.88 percent FSB infestation, no farmers found it necessary to spray even a single insecticide application for FSB control and almost all farmers were experiencing at least a two-fold increase in marketable yield. Final field results on crop performance and socio-economic benefit will be presented by BARI in September 2015. BARI currently has five additional Bt eggplant varieties under development. It is expected that two of the varieties will be approved for propagation and seed multiplication for the next growing season. The other three Bt eggplant varieties continue to be bred for genetic identity for trueness-to-type.

ABSPII's successful partnership with BARI is a highly significant breakthrough since the release of bt eggplant is the only public deregulation of a GM crop since papaya ringspot virus resistant papaya was released in Hawaii. And, it is the first GM food crop to be deregulated in a developing country. This significant event is due to the strong political will of the Government of Bangladesh to establish food security for the country of Bangladesh and their trusted partnership with Cornell University.

The efforts to commercialize Bt eggplant could easily be lost without further support from USAID Bangladesh. The Government of Bangladesh and BARI are not in a position to provide the expertise needed to move the product beyond the field observation phase. The true cost of moving the product forward is actually orders of magnitude greater than the amount of money that has already been spent to bring the product through deregulation. ABSPII is hopeful that the Government of Bangladesh will provide for most of the cost, but the training for seed multiplication, inventory management, distribution and stewardship will require significant inputs from ABSPII/USAID. We are hopeful that someone in USAID will realize how important it is that BARI continue to be supported financially so as to prevent a major product failure and embarrassment for both USAID and Cornell University.

Philippines: Our ABSPII partner at the University of the Philippines Los Baños-Institute of Plant Breeding (UPLB-IPB) developed Bt eggplant varieties and hybrids for the Philippine market. During 2010-2012, multi-location confined field testing was conducted on the UPLB Bt eggplant open pollinated (OP) lines and F1 hybrids containing event 'EE-1' to generate field data under local conditions for environmental safety assessment, National Seed Industry Council (NSIC) variety accreditation and FPA registration. The specific objectives of the trials were: 1) to evaluate the field efficacy of UPLB Bt eggplants containing 'EE-1' against the target pest, eggplant FSB; 2) to assess the potential adverse effects of UPLB Bt eggplants containing 'EE-1' to non-target organisms; 3) to identify intended and unintended changes in UPLB Bt eggplants containing 'EE-1' and assess its potential to become plant pests; and 4) to determine the amount of Cry1Ac expressed in Bt OP lines and F1 hybrids.

Based on the extensive set of data obtained from 24 successfully completed confined field trials, the specific objectives of the multi-location field trials of Bt eggplants OP lines and F1 hybrids containing event 'EE-1' were successfully achieved. Overall, the comparative assessment studies between Bt eggplants OP lines and F1 hybrids containing event 'EE-1' and their conventional non-Bt eggplant comparator genotypes demonstrated the following results consistently in all test sites:

1) Bt eggplants OP lines containing event 'EE-1'grown without EFSB-insecticide spray exhibited very high field efficacy (up to 100 percent) against the target insect pest, eggplant FSB, even under the most severe eggplant FSB pest pressure encountered in Pangasinan. Significantly lower damages in the shoots and fruits, fewer eggplant FSB larvae/plot and shorter larval feeding tunnels were detected in Bt OP lines vs. non-Bt comparators. Similar results were obtained in hybrid trials also grown without eggplant FSB insecticide spray, despite the hybrids being heterozygous for *cry1Ac* gene from 'EE-1'. The field efficacy of UPLB Bt F1

hybrids remained very high (100 percent) relative to the non-Bt hybrid near-isolines and commercial check.

2) No viable F1 progeny or offspring were produced from any of the few surviving EFSB larvae collected in the field from Bt eggplant OP lines and hybrids from two trial sites (Laguna and Pangasinan). Results indicate high-dose effect of Bt eggplants and the possibility of using a high-dose/refuge insect resistance management (IRM) strategy to delay the potential development of resistance in eggplant FSB.

3) Univariate and multivariate analyses of data from season-long monitoring of non-target organisms (NTOs) in all trials conducted indicated no significant adverse impacts of Bt eggplant OP lines and F1 hybrids on species abundance, diversity and community dynamics, particularly of beneficial non-target arthropods (NTAs). Principal Response Curve (PRC) analyses showed no statistically significant negative impact of UPLB Bt eggplants on non-target arthropod communities through time. The abundance of the taxa observed varied among sampling dates but not between crop types (Bt vs. non-Bt). As expected, most secondary lepidopteran pests were negatively affected by Bt eggplants. Some generalist predators were more associated with non-Bt eggplant due to the presence of more preys/hosts. Only coccinellids were present in all the trials across sites, and so may be used as an indicator species in post-introduction monitoring of Bt eggplant.

4) All UPLB Bt eggplant OP lines and hybrids out-yielded the non-Bt comparators in all trial sites. Significantly higher undamaged fruit yield were obtained in Bt eggplant OP lines and hybrids compared to the non-Bt comparators particularly under severe pest pressure. The highest yield advantage recorded was more than 7000 percent in Pangasinan over the non-Bt commercial check variety. Higher yield performance in Bt OP lines vs. the non-Bt comparators is the altered trait or intended change expected as a result of the incorporation of event 'EE-1'.

5) Comparable horticultural and phenotypic characteristics, and reactions to natural field incidence of pest and diseases were observed between Bt and the non-Bt comparators. When significant differences were observed, the magnitude of difference observed was small, and the traits observed in Bt eggplants fell within the range of natural variation observed in the cultivated eggplant species. No volunteer eggplant seedlings were found within the 60-day fallow observation period. The results suggest that there were no biologically significant unintended changes in UPLB Bt eggplant OP lines and F1 hybrids as a result of the incorporation of event 'EE-1'.

6) Results of the BPI-recommended additional studies on the effects of weekly spraying of recommended rate of flubendiamide, a lepidopteran-specific insecticide, demonstrated that

the percent efficacy of the unsprayed vs. sprayed Bt lines remained very high (100 percent) indicating that eggplant FSB insecticide spraying may not be necessary in Bt eggplant production. Furthermore, lower eggplant FSB crop damage and higher yield were obtained in unsprayed Bt vs. flubendiamide-sprayed non-Bt eggplant comparators, but with no significant adverse effect on non-target arthropod communities. The results indicate that Bt eggplant containing 'EE-1' is potentially a more efficacious, environmentally benign and profitable alternative to the current practice of excessive use of chemical insecticides in eggplant production.

7) In the Ratoon crop studies, with and without weekly application of insecticide flubendiamide, the field efficacy of unsprayed Bt eggplants remained very high compared to non-Bt eggplant comparators. There were no significant differences in NTO communities detected by PRC analysis between the two crop types.

8) The Cry1Ac protein expression was found to be variable between different plant parts and at different stages of development. The highest levels of Cry1Ac expression was detected in the terminal leaves followed by the flowers, fruit flesh, fruit skin, stem and roots. Genotype-independent season-long variation of the Cry1Ac protein levels was also observed. Results indicate that the levels of Cry1Ac expression in the terminal leaves and fruits of Bt eggplant OP lines and F1 hybrids remained sufficiently high even under high eggplant FSB pest pressure, resulting in reduced damage (100 percent efficacy) and fecundity (no viable eggs or offspring). Collectively, the results obtained from two-year multi-location field testing support the following conclusions, which are consistent with results obtained for Bt eggplants in India and the documented environmental safety of GM crops containing Bt Cry1Ac:

   a) The UPLB Bt eggplant OP lines and F1 hybrids containing event 'EE-1' possess a novel trait that provided outstanding control of eggplant FSB making them superior to the conventional counterparts and the check, particularly when the pest pressure is high;

   b) The UPLB Bt eggplant OP lines and F1 hybrids containing event 'EE-1' have no demonstrated adverse effects on NTOs, particularly on beneficial non-target arthropods;

   c) The UPLB Bt eggplant OP lines and F1 hybrids, except for the intended change (i.e. undamaged fruit yield) have comparable horticultural and phenotypic characteristics and reactions to eggplant diseases and other pests compared to that of conventional eggplant controls, and therefore, are not likely to pose any increased plant pest risk;

   d) The UPLB Bt eggplant OP lines and F1 hybrids have sufficient amount of Cry1Ac expressed in relevant plant parts and demonstrated efficacy resulting in diminished

reproductive capacity of eggplant FSB suggesting the possibility of using a high-dose/refuge IRM strategy for Bt eggplant deployment in the Philippines.

There were no unexpected biosafety-related effects observed. All conditions in the Biosafety Permit for every trial in every testing site were complied with except in two incidents. The first incident involved the field trial in UP Mindanao, Davao City. The trial was abruptly terminated due to a cease-and-desist order issued by then-Mayor Sara Duterte on December 15, 2010. This was reported to DA-BPI. The cease-and-desist order was carried out by City Agriculturist Mr. Avila on December 17-18, 2010, under protest from UP Mindanao officials who were on the premises at that time. All plant materials were uprooted and buried within the trial site following biosafety procedures. The site was kept locked and unused during the 60-day fallow period. No other Bt eggplant trial was held in UP Mindanao thereafter. A complete report of the incident was submitted to DA-BPI.

The second incident involved the OPV Trial 2 conducted in Laguna. Greenpeace personnel and others led by Daniel Ocampo trespassed and forcefully entered the UPLB Bt eggplant field trial site on February 17, 2011, and uprooted test plants and all pollen trap plants bordering one side of the trial plot. The incident was immediately reported to DA-BPI and the proponent requested that the experiment be terminated earlier than scheduled because of absence of pollen traps (the presence of pollen traps on all sides of the trial site was part of the Biosafety Permit condition). A criminal case was filed against Greenpeace and others at the Municipal Trial Court in Bay, Laguna and the case is still under trial.

An unexpected setback for the Bt eggplant product in the Philippines occurred when a petition for a Writ of Kalikasan (Writ of Nature) with Temporary Environmental Protection Order (TEPO) against the Bt eggplant field trials was filed in the Supreme Court of the Philippines by Greenpeace et al in April 2012. The TEPO was not granted and the case was given to the Court of Appeals (CA) for trial. The CA ruled with finality to stop the Bt eggplant field trials on October 2013. An appeal was filed in the Supreme Court and the case is on-going. No field trials were stopped by the court action because all Bt eggplant field trials were already completed. The ruling was limited and has no impact on the eventual application for commercial release, i.e. secure biosafety approval and FPA registration. All of the multi-location field trials have been completed but application for commercial propagation is delayed pending the resolution of a petition filed in the Philippine Supreme Court. In addition to the Bt hybrids, two non-Bt hybrid counterparts were approved for release by the NSIC variety accreditation of the Philippines in 2013. These are the first eggplant hybrids released by a public sector institution in the Philippines. We anticipate submitting a regulatory dossier to the regulatory authorities in the Philippines before the end of 2015 and expect approval for propagation in 2016.

A significant effort was invested in outreach and communications on Bt eggplant in the Philippines, and it probably is one area that will leave an enduring impact on project partners. ABSPII conducted extensive and systematic capacity building activities in communicating agri-biotechnology and Bt eggplant technology for collaborators, IBCs and regulators from the national and regional offices with focus on field trial sites. At the same time, the project established a network of support groups for the technology and conducted social mobilization and alliance building activities, with focus on priority field trial regions/sites and national competent agencies and leadership. As a result of these efforts, no less than 19 formal resolutions were issued by key local government units and farmer organizations in support of Bt eggplant, and the overall media coverage about Bt eggplant has shifted from an initial negative to a more positive one. The project likewise developed key messages on biotechnology and Bt eggplant, and packaged and disseminated information through multi-media and social media platforms and developed local language translations of Bt eggplant and other biotechnology information materials and feature articles, and distributed technology-specific information. Through these exhaustive efforts, ABSPII is confident that our partners in the Philippines will prevail and that Bt eggplant will become part of a sustainable and cost effective pest management option for Filipino eggplant growers.

Late Blight Resistant Potato: India, Bangladesh and Indonesia

Product and Participating Countries	Partnering Institutions	Technology	Current Status
Late Blight Resistant Potato Bangladesh, India, Indonesia	• Bangladesh Agricultural Research Council (BARI) • BARI Potato Research Center • Central Potato Research Institute, India • Indonesian Agency for Agricultural Research and Development • Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development • Indonesian Vegetable Research Institute • International Potato Late Blight Testing Program, Mexico • International Potato Center, Peru • Michigan State University, USA • Sathguru Management Consultants, India	• A gene isolated from a wild potato species (*Solanum bulbocastanum*) called *Rb* has been found to be responsible for conferring high levels of resistance to late blight disease. • Unfortunately, this wild potato is genetically distant enough that it cannot be conventionally crossed with commercial potato. • The *Rb* gene has been cloned and transformed into a commercial potato variety and an event	• Partners in India have conducted efficacy trials and have selected 5 lines of Kufri Jyoti hybrids for regulatory trials. • Partners are currently working towards carrying out Environmental Risk Assessment studies and developing a regulatory dossier. • In Bangladesh, BARI scientists have concluded the efficacy trials and have selected 6 hybrid lines of Diamant variety for regulatory trials. • Indonesia has concluded Environmental Risk Assessment studies and is in the process of developing a regulatory dossier • The RNAi technology from Venganza Inc. is being considered as a second technology to improve the durability of the resistance technology for the longer term. • Indian and Indonesian scientists received

	• University of Wisconsin, USA • Venganza Inc., USA	has been selected that shows high levels of resistance to late blight disease. • ABSPII partners are using this event to conventionally breed this technology into locally popular varieties.	training in potato transformation for the RNAi construct at Cornell University. • Indonesia has completed multi-location field trials, food and feed biosafety studies and environmental impact studies required for commercial release.

Potato is an important vegetable crop in India, Bangladesh and Indonesia, where it is primarily grown by resource-poor farmers. A highly nutritious food that provides many essential vitamins, minerals and amino acids, potato is an important supplemental source of nutrients and calories for people living on rice-dominant diets. Potato cultivation is plagued by a fungus (*Phytophthora infestans*) that causes late blight (LB) disease. This disease ranks among the most serious crop diseases worldwide and spreads extremely fast. An entire crop can be destroyed within one to two weeks under certain conditions. Controlling this disease is essential for resource-poor farmers who depend on potato for basic nutrition. Development of crop resistance to late blight by conventional breeding methods has proven extremely difficult. To fight the disease, farmers who can afford fungicides are forced to apply them repeatedly. Excessive use of fungicides, as many as 45 applications per season in India, Bangladesh and Indonesia, poses serious environmental and health risks and greatly reduces farmers' profits. Fungicide applications often are ineffective due to pathogen resistance to many of the common fungicides used to mitigate late blight.

A wild potato species (*Solanum bulbocastanum*) possesses a gene, called *Rb*, which confers high levels of resistance to late blight disease. Unfortunately, this wild potato is genetically distant enough from cultivated potato that attempts to transfer the gene into domesticated potato varieties using conventional breeding have not been successful. The *Rb* gene was cloned from *S. bulbocastanum* and transformed into the popular potato variety Katahdin by researchers at the University of Wisconsin. Genetically engineered Katahdin potatoes demonstrated high levels of late blight resistance (LBR) in field trials conducted in Mexico, Wisconsin, and Minnesota. ABSPII commissioned the LBR Potato Project in 2005. The University of Wisconsin Alumni Research Foundation (WARF), with the help of ABSPII's partner, Sathguru Management Consultants Pvt. Ltd., donated two LBR Katahdin events, SP951 and SP904, to partner scientists in India, Bangladesh and Indonesia.

Our partner institutions crossed both events into farmer preferred potato varieties, Atlantic and Granola, in Indonesia, Diamant in Bangladesh and numerous varieties in India including the popular potato Kufri Jyoti variety. Based on field trial efficacy trials conducted from 2008-2012

in Indonesia and Bangladesh under natural fungal infestation conditions, event SP951 proved superior to SP904. Early in the project, efforts were made to produce new transformation events directly in the popular varieties using an updated *Rb* T-DNA vector, but SP951 proved superior over all the new events and a decision was made to focus on breeding rather than searching for events with efficacy superior to that of SP951.

Overall, event SP951 demonstrates significantly reduced leaf tissue infection (11-25 percent) compared to non-transgenic controls (60 to >90 percent) resulting in an 80-90 percent reduction in fungicide applications. The visible reduction in foliar damage in SP951 and hybrid clones was quite remarkable and clearly commercially viable so a decision was made to move forward with product development and regulatory activities. Our original product profile for the late blight resistance and the basis for ex-ante socio-economic analysis was done assuming a 45 percent reduction in fungicide application. It is anticipated that the *Rb* gene technology will become an important component in an integrated pest management system for late blight disease in potatoes grown in South and Southeast Asia.

This project represents a model system for North-South and South-South partnership and cooperation. With the support of ABSPII, scientists from India, Bangladesh, and Indonesia were well trained to conduct field tests, perform product development activities, conduct studies for regulatory approval and undertake outreach efforts. Early in the project, scientists from India and Bangladesh were trained in potato transformation techniques and molecular characterization at the University of Wisconsin, Madison in year 2005.

Central Potato Research Institute, India (CPRI) scientists received substantial in-country resource support to facilitate biosafety, food safety and environmental safety validations and technical guidance from regional cooperation among Asian partners. For example, Indonesian scientists shared transformation protocols for evaluation in India. Similarly, Wisconsin provided relevant laboratory protocols to both Indian and Indonesian partners to refine transformation protocols, allowing them to succeed in regenerating transformed materials.

Scientists from BARI, Bangladesh engaged in intensive training on transformation and breeding techniques with the help of University of Wisconsin and Indonesian partner institutions. Local capacity of Bangladesh scientists in intellectual property assessment, technology transfer and licensing arrangements was increased with the support of other partner institutions and ABSPII resource persons. In 2013, scientists from India and Indonesia were provided additional training at Cornell University on transformation of RNAi technology for late blight resistance.

Since the *Rb* gene produces an intractable protein, it will not be possible to conduct studies on purified protein to establish biosafety. In this case, our partners will build a case around "weight of evidence" on the LBR potato product. An extensive literature search will be conducted and evidence showing that all plants produce entire families of R genes in response to infection by different disease pathogens. For potato, the main focus will be on establishing a strong history of safe use.

Current Project Status by Country and Continued Efforts Required for Successful Product Adoption

India: The Central Potato Research Institute (CPRI), in Shimla, India, was commissioned by ABSPII to develop LBR potato varieties popular in India. Select lines were crossed with the popular Indian Kufri Jyoti and Kufri Bahar potato varieties among many others. Based on potato variety selection and LBR efficacy and other potato product development parameters, several candidate hybrid varieties of Kufri Jyoti were selected for further trials.

More specifically, the transgenic Katahdin event SP951 harboring the *Rb* gene was used as male parent to cross with the popular Indian potato cultivars *viz*., Kufri Bahar (KB) and Kufri Jyoti (KJ). Populations of 271 F_1 hybrid lines (KB × SP951 (87) and KJ × SP951 (184)) were screened for integration of *Rb* transgene through PCR. Hybrid lines positive for the *Rb* gene, were further phenotyped for late blight resistance by challenge inoculation. Disease response based on area under the disease progress curve (AUPDC) of different hybrid lines varied from near immunity to complete susceptibility. High degree of resistance (<25 percent infection) was observed in 88.6 percent of KJ × SP951 derived hybrids, whereas the level of resistance in KB × SP951 (58.4 percent) derived hybrids was of low order (>25 percent infection). Therefore further progress was limited to Kufri Jyoti hybrid lines.

Hybrid lines positive for *Rb* gene were further phenotyped for late blight resistance by challenge inoculation under field conditions. All selected lines showed resistance response to late blight under field conditions (field evaluation of male parent and hybrid lines in 2009, 2011 in Modipuram, and 2012 in Shimla). In 2013, ABSPII partner CPRI selected the best five lines for further evaluation for efficacy in terms of yield as well as blight resistance under containment facility. Quantitative real time PCR expression analysis demonstrated that high levels of resistance were associated with induction of *Rb* transcripts. Furthermore, the sequence of the *Rb* gene in SP951 was compared with the known sequence of the *Rb* gene and found to be identical. In 2014-15, molecular characterization studies were done which confirmed the insert number of the kanamycin resistance gene NPTII and co-integration of the gene in *Rb* hybrids genome.

Further, event specific primers were developed for insertional characterization of *Rb* gene in SP951 and Kufri Jyoti hybrid lines. Analysis of left and right border regions in SP951 demonstrated that the insertion of the *Rb* gene did not disrupt any functional genes nor did it create new open reading frames that encoded proteins with a significant match to non-redundant sequence database queried by the BLASTP program. To elucidate the downstream action of *Rb* genes resistance to *Phytophthora infestans*, we performed a comprehensive transcriptional analysis using cDNA microarrays, containing TIR transcripts and 61031 unigenes. Leaves of Kufri Jyoti *Rb* transgenic resistant potato lines were inoculated with *P. infestans* and sampled at four time points ranging from 0 to 96 h after inoculation. A total of 2827 *P. infestans*-responsive genes were identified in all the selected resistant lines.

Currently, our partners at CPRI are focusing on developing the food safety dossier, BRL-1 trial and varietal release. The prevailing moratorium on field testing of GM crops in India has set back the progress of the project. Multi-location field testing of any GM crop requires approval from individual states for field testing of GM crops and many of the potato growing states have restrictive policies on GMO testing. Despite being unable to conduct confined field trials in India, CPRI collaborated with BARI and ICABIOGRAD to develop a unified approach to generate biosafety and environmental data required for the regulatory dossier. ABSPII hired two professional regulatory consultants as central resource people for guidance on biosafety and environmental evaluation and regulatory dossier development. Current work on the LBR potato project is focusing on developing the food safety dossier, product commercialization, seed distribution and product stewardship. In India, substantial in-country resource support has been mobilized to facilitate biosafety, food safety and environmental safety validations for the deregulation and eventual commercial release of the product.

Indonesia: The typical fungicide spray program to control late blight in conventional potatoes in Indonesia can result in ~20-45 applications per season. Data from several years of field trials indicate that event SP951 provides excellent late blight disease control for the majority of the growing season, without any fungicide application. As the potato plants mature and redistribute energy from foliar growth to tuber filling, above-ground foliar disease increases. Thus, it is anticipated that a few late season fungicide sprays will be needed to maintain plant health in varieties produced from SP951. Product development work in Indonesia has progressed well and there is enthusiasm from the Government of Indonesia to commercialize the *Rb* expressing LBR potato. Farmer field days were held at all of the field trial locations as a communications and outreach effort to educate farmers on the technology. We experienced minimal interference in Indonesia from anti-GM activists. The final round of multi-location field trials, compositional analysis and protein toxicity testing was completed in 2013-2014. The

ABSPII team and partners in Indonesia bulked up seed stocks. All of the environmental and food safety studies have been completed and the regulatory dossiers will be submitted to regulatory authorities in Indonesia in 2015. Commercial release and propagation of *Rb* potato is expected in 2016.

Food safety studies that include compositional analysis, toxicity, and bioinformatics searches for allergenicity have been completed. The genetic materials used in the food safety study were genetically engineered (GE) potato Katahdin SP951 and non-GE as well as *Rb* expressing hybrid lines. The objective of compositional analysis study was to conduct compositional and nutritional analysis of GE potato Katahdin SP951 and non-GE as well as hybrid lines. Samples of tubers used in this analysis study were GE and non-GE tubers which were harvested from CFT Banjarnegara, Central Java (dry season, 2012) and CFT Pangalengan, West Java (wet season, 2013), consisted of tubers of six selected hybrid clones and four controls (GE Katahdin SP951, non-GE Katahdin, Granola, and Atlantic). Parameters of compositional and nutritional for the analysis consisted of key nutrient (dry matter, protein, starch, total fat, dietary fiber, crude fiber, minerals, sugar and amino acids), ascorbic acid, dehydroascorbic acid, and total glycoallkaloids. Result of the study demonstrated that the composition and nutrition of GE potato Katahdin SP951, non-GE Katahdin and hybrid lines of GE potato Katahdin SP951 and Granola/Atlantic in CFT Banjarnegara and Pangalengan were substantially equivalence. Total glycoalkaloids of GE potato Katahdin SP951 and the control Katahdin as well as the hybrid lines in CFT Pangalengan were also equivalent.

The toxicity studies comprise of bioinformatics of *Rb* protein to known toxin and oral acute toxicity of juice and powder of potato tuber to mice. The bioinformatics analysis was done to assess the homology of *Rb* protein with other toxins or allergins. Bioinformatics analysis was carried out using Emboss Transeq program from EMBL-EBI website to translate *Rb* gene to its amino acid sequence, *Shuffle Protein Program* to obtain randomized amino acid sequence, and *BLASTP search program* to find proteins with similarity and homology, and also BLAST *Suite-2 sequences* to analyze the homology between two or more alignments.

The results showed that homology occurred with the e-value of lower than 0.22 and further analysis indicated that *Rb* protein contains no significant sequence homology to any protein known as toxin in the Entrez protein database of NCBI. Therefore, it can be concluded that *Rb* protein is not toxic or allergenic. As mandated by the Government of Indonesia, oral acute toxicity of GE potato Katahdin SP951 was compared with the control non GE Katahdin and their hybrid lines. The results showed that the administration of juice and powder from tuber of GE potato Katahdin SP951, control non GE Katahdin and hybrid lines in all treatments (0, 1.25, 2.5, 5, 10, and 21 g/kg body weight) for up to 14 days did not show any negative effects on mice, as

indicated by no mortalities. Observation on body weight showed that mice (females and males) in the GE potato Katahdin SP951 group were not statistically different compared to controls non-GE Katahdin at day 8 in all of treatment of potato juice and powder. Similarly, the bodyweights of mice in the hybrid lines group also were not statistically different compared to GE and non-GE potato at day 8 in all of treatment of potato juice and powder. Observation on clinical signs (red and watery eye, bloody nose, shaking body, weak body, difficult to breath, and diarrhea) showed that there were no clinical signs detectable in all treatments after 14 days of testing. This result indicated that the administration of potato juice and powder from GE potato Katahdin SP951, non-GE Katahdin and the hybrid lines showed no effect on the clinical signs of mice.

The allergenicity studies consist of bioinformatics and protein stability to digestibility through simulated gastric fluid (SGF) and simulated intestinal fluid (SIF). Bioinformatics analysis and SGF and SIF were carried out to determine whether the protein of GE potato Katahdin SP951 has potential as an allergen. Bioinformatics analysis was performed using the program FASTA and BLASTP Alignment through the data of protein sequence comparator available on Allergen Online and NCBI database, while the studies on SGF analysis used pepsin enzyme and SIF used trypsin enzyme. The results of bioinformatics analysis showed that the protein encoded by the *Rb* gene in GE potato Katahdin SP951 has no amino acid sequence that is homologous to the allergen protein, therefore it can be concluded there is remote potential to cause an allergy.

Results of the analysis of SGF and SIF for testing of allergenicity for GE potato Katahdin SP951 and hybrid lines indicated that the total protein of GE potato Katahdin SP951 and hybrid lines degraded less than 5 minutes of incubation after being treated by enzymes of degrading protein. The results of SGF and SIF analysis results indicated that the total protein of GE potato Katahdin SP951 and hybrid lines are fully digested in the human digestion system.

Since the data of food safety studies have been completely analyzed, the dossier for food safety risk assessment has been finally prepared. The application of food safety risk assessment will be signed by the director of ICABIOGRAD and, together with the dossier, sent to the National Agency of Food and Drug Control (NAFDC). We expect that by the end of 2015 there will be food safety recommendation by IBTT to the Indonesian Biosafety Committee. All of these more recent activities have been funded by the Government of Indonesia (GOI). Due to the limitation of GOI funding for 2015, the environment safety risk assessment will be applied for next year (2016). The dossier for environment safety risk assessment will be revised and prepared. The dossier and application of environment safety risk assessment will be sent to the Ministry of Agriculture and Ministry of Environment and Forestry in 2016. Our partners in Indonesia were accustomed to US-AID's lack of commitment to the project and continued to work closely with

the Indonesian Government from the inception of the project. The GOI is strongly committed to achieving food security for its large population and see late blight resistant potato as one of the major means to reach that goal.

Bangladesh: The *Rb* gene was successfully introgressed into the farmer-preferred cultivar Diamant. Field trials were conducted since 2008 (two locations each in 2008, 2009, 2010, six locations each in 2011, 2012, 2013 and 2014). The *Rb*-expressing hybrids exhibited excellent foliar control of late blight and had markedly increased yields over the non-transgenic Diamant variety. Scientists from BARI established the efficacy of the LBR potato product through multi-location field trials (MLFT) in Bogra, Comilla, Chittagong, Gazipur, Jessore and Rangpur in Bangladesh during FY 2013–14. Six hybrids of the genetically modified Diamant line performed exceptionally well against late blight infection and were further tested for establishing substantial equivalence between transgenic and control lines during FY 2014-15 regulatory trials. Among the six hybrids, no distinguishable variation was shown on morphological characters between transgenic and non-transgenic counterparts. Tuber quality parameters have yet to be established, but the tubers appear similar. The environmental studies suggested no difference in non-target insect populations and no adverse effect on beneficial insects.

In 2014, a trial to establish substantial equivalence and non-target affects under confined field conditions (CFT) was conducted at six locations (Gazipur, Bogra, Jessore, Rangpur, Comilla and Chittagong) of BARI with six transgenic RB hybrid clones of potato. The clones were: D-951 (2), D-951 (3), D-951 (12), D-951 (13), D-951 (137) and D-951 (304). Non-transgenic BARI Alu-7 (Diamant) was used as the check. Seed tubers were planted from December 2-8, 2014. The experimental plot was laid out in RCB design with three replications. As this year's trial was regulatory perspective, so non transgenic Diamant (control) was sprayed alternating with fungicides Secure (0.1%) and Dithane M-45 (0.2%) from initiation of late blight to keep it free from the disease. Late blight appeared with different degrees of severity in five (Gazipur, Bogra, Jessore, Comilla, Rangpur, and Comilla) out of six locations. There was no late blight infection at Hathazari, Chittagong. The initiation of disease symptoms was detected at 35 to 70 days of plant age in border crop Diamant where no fungicide was applied. In each location non transgenic border crop Diamant was damaged 100 percent while hybrid lines performed well to late blight having distinguishable lower AUDPC value. AUDPC value was less than 100 in all five locations except Bogra. Many insect pests and beneficial insects (aphid, ladybird beetle, cut worm, whitefly etc.) were observed in all RB hybrid lines along with the control variety indicating RB hybrid lines had no harmful effect on insects. PLRV, PVY and PVX were observed sporadically. Bacterial wilt, soft rot, stem canker and dry rot were observed irregularly in different locations. Common scab was observed in all the hybrid lines as well as the control, Diamant. Morphological and tuber characteristics were similar in both transgenic lines and non-

transgenic Diamant. Yield varied to some extent over locations in both transgenic lines and non-transgenic Diamant.

For submission of a regulatory dossier to government officials in Bangladesh in 2016, one additional environment study on gene equivalency must be conducted in the 2015-16 growing season. The trial will include four hybrid *Rb* Diamant SP951 hybrids and Katahdin as a control. Two hybrids have been discarded due to unacceptable virus susceptibility. The best yield hybrid with the best cooking characteristics will be used as criteria in narrowing down one or two lines for release. Ideally, one line will be selected for release. As of now, Diamant 951 (137) is the best performing hybrid based on efficacy and trueness-to-type. Currently the LBR potato is the only known source of technology in the world that is successfully demonstrated in the field to mitigate season-long late blight in potato. All other technologies are several years away from even basic field validation. The predominant focus of BARI scientists for LBR potato development in the next 36 months will be to conduct regulatory trials and secure approval of the biosafety dossier for release of *Rb* transgenic lines in Bangladesh. LBR potato developed by BARI is a completely public sector effort with technology from a public institution and the whole development effort pursued by a public institution.

Deregulation Status and Commercialization of GM Potato

In 2013-2014, CPRI focused on securing a consensus decision from the Review Committee on Genetic Manipulation (RCGM) to pursue an alternate approach to the evaluation of the pure protein because of the intractable nature of R proteins. CPRI is collaborating with BARI and ICABIOGRAD to draw a unified approach to generate the biosafety data and is guided by the ABSPII central resource person for biosafety evaluation. The prevailing moratorium in India has set back the progress of the project, as the multi-location field testing mandates approvals from individual states for field testing of GM crops. Many of the states have restrictive policies on GMO testing. It is not known when more field trials can be conducted.

The environmental safety risk assessment dossier for LBR potato event SP-951 will comprise genetic information and environmental safety information. Genetic information includes element genetic, source of *Rb* gene, plant transformation methods, genetic stability, and absence of vector backbone data. The vector backbone study is under progress. Environmental safety information will consist of intended and unintended effects.

Food safety information will consist of substantially equivalent (compositional analysis), toxicity, and allergenicity assessment. ABSPII will conduct compositional analysis, estimation of

glycoalkaloids and antinutrients, toxicity and allergenicity studies based on the OECD consensus document on potato.

A second generation technology for generation of a more durable late blight resistance phenotype will be developed by stacking RNAi constructs into the best performing *Rb* potato. The RNAi constructs have been developed by ABSPII's partner, Venganza, Inc. Gene transformation was conducted in the potato variety Katahdin and Desiree as standalone events and as a stacked trait in *Rb* potato SP951 event. Scientists from India and Indonesia have received training in potato transformation for the RNAi construct at Cornell University.

ABSPII Products Initiated but Discontinued for Various Reasons

Product and Participating Countries	Partnering Institutions	Technology	Current Status
Papaya Ringspot Virus Resistant Papaya Philippines	• Department of Science and Technology – Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development, Philippines • International Service for the Acquisition of Agribiotech Applications, Philippines • Monsanto • Southeast Asian Regional Center for Graduate Study and Research in Agriculture, Philippines • University of the Philippines Los Baños • University of the Philippines Los Baños Foundation • Virginia Polytechnic Institute and State University, USA	• Most viruses are encapsulated by coat proteins that act as a protective cage for the virus. • It has been well documented in many plants that introduction of a specific viral coat protein can render the plant resistant to the target virus. • A papaya ringspot virus (PRSV) coat protein gene is being transformed into local papaya varieties, essentially vaccinating the plants against PRSV. The gene sequence being used is specific for the virus that occurs in the Philippines.	• Based on greenhouse and confined field trial data, transgenic plants have been generated that show high levels of resistance, but not immunity, to PSRV. • Research activities on the plants possessing the coat protein technology and others employing the use of double stranded RNAi technology are being continued UPLB in an effort to produce plants with durable resistance to the virus. • ABSPII also supported the capacity building of the UPLB scientists in the area of biosafety regulation, particularly in the conduct of the first confined field trial of a tree crop.
Drought and Salinity Tolerant (DST) Rice Bangladesh, India	• Bangladesh Rice Research Institute (BRRI) • Central Soil Salinity Research Institute, India • Cornell Research Foundation, USA • Directorate of Rice Research, India • Government of India, Department of Biotechnology • International Center for Genetic Engineering and Biotechnology, India • Sathguru Management Consultants, India • Tamil Nadu Agricultural University, India	• A drought and salt tolerant technology developed by the late Prof. Ray Wu at Cornell University is being tested in transgenic rice to assess the plant's abilities to tolerate high-stress growing conditions. • The technology involves manipulating genes required for synthesis of the naturally occurring sugar trehalose.	• This project has been dropped from ABSPII in India and Bangladesh due to cessation of funding. • However, the biomaterial developed by International Center for Genetic Engineering and Biotechnology, India under the project is in advanced stages and will continue product development efforts by seeking national funding and, if possible, collaborating with private sector partners. • In Bangladesh, scientists from BRRI have received training from International Center for Genetic Engineering and Biotechnology, India for rice transformation and will be taking the product development efforts forward by seeking national funds.

Product and Participating Countries	Partnering Institutions	Technology	Current Status
Multiple Virus Resistant Tomato Indonesia, Philippines	• Bogor Agricultural University, Indonesia • East West Seeds, Philippines • Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development, Indonesia • Indonesian Vegetable Research Institute • International Service for the Acquisition of Agribiotech Applications, Philippines • University of the Philippines, Los Baños, Philippines • Virginia Polytechnic Institute and State University, USA • World Vegetable Center, Taiwan	• Coat protein technology has been successfully used to develop tomatoes with resistance to the cucumber mosaic virus (CMV). No commercial tomato varieties are resistant to CMV. • Tomatoes with naturally occurring resistance to tomato leaf curl viruses have been identified in several wild tomato species and transferred to elite tomato breeding lines by the World Vegetable Center. • Thus, the aim of this project is to use a combination of a transgenic approach for CMV and marker – assisted breeding for tomato leaf curl virus and tomato yellow leaf curl virus to confer resistance to multiple viruses into locally adapted varieties preferred by farmers.	• Funding for this project in the Philippines and Indonesia was discontinued when attempts to develop effective events were unsuccessful. • In Indonesia, it has been found that when the technology is crossed into other varieties with some resistance to the viruses, varieties can be developed that are very resistant to cucumber mosaic virus. • In the Philippines, efforts are underway under alternate funding to produce tomatoes that are resistant to cucumber mosaic and other tomato virus complexes.
Tomato Virus Resistance for West Africa Mali	• Centre pour le Developement de l'Horticulture/Institut Senegalais de Recherche Agricole • Institut National de Recherche Agronomique du Benin • Institut National de Recherche Agronomique du Niger • Institut Togolais de Recherche Agricole, Togo • Institut d'Economie Rurale, Mali • Institut d'Etudes Environnementales et de Recherches Agricoles, Burkina Faso • National Bureau of Plant Genetic Research, India • University of California, Davis, USA • World Vegetable Center, Taiwan	• The primary effort of this project was to identify existing germplasm that was resistant to tomato yellow leaf curl virus (TYLCV) and cross it with transgenic tomatoes that would be developed for resistance to potyviruses, which were believed to be a serious challenge to tomato production in West Africa.	• ABSPII decided to discontinue funding research on this project when screening tests indicated that potyviruses were no longer a major threat to West African tomato production because of the severity of the TYLCV. TYLCV was devastating crops before potyviruses had a chance to develop.
Tobacco Streak Virus Resistant Groundnut India	• Acharya N.G. Ranga Agricultural University, India • Donald Danforth Plant Science Center, USA • International Crops Research Institute for the Semi Arid Tropics (ICRISAT), India • National Bureau of Plant Genetic Research, India • Sathguru Management Consultants, India	• The tobacco streak virus coat protein gene from the virus that occurs in India was transformed into a local variety of groundnut essentially vaccinating it against the virus through its own defense mechanism.	• Given that this product was still in the early research phases, not in a product development phase, ABSPII elected to discontinue funding. In the interim, ICRISAT has continued to conduct research and product development activities on this product and the technology is showing promising results.

Listed above are some of the projects originally initiated by ABSPII but terminated for various reasons. The reasons for termination included lack of sufficient efficacy from the technology being employed to see the products through commercialization (Tobacco Streak Virus Resistant Groundnut in India and Multiple Virus Resistant Tomato in Indonesia and the Philippines). The Tomato Virus Resistance for West Africa product was terminated because another virus introduced to West Africa was so serious a problem that the potyvirus problem became insignificant. The papaya ringspot resistant papaya product for the Philippines and the drought and salinity tolerant rice product for India and Bangladesh were discontinued due to lack of funding support from ABSPII.

Even though these product lines were discontinued, there are some very valuable attributes from these projects that should be mentioned. For example, Dr. Sneh Lata Pareek from ICGEB, India travelled to Cornell University for training on molecular characterization and transformation studies in rice. She imparted training to four scientists from BARI, Dhaka on molecular characterization and transformation studies in rice. Her work on the DST rice project continues with some very promising events. The limitations have been the inability to perform confined field trials to establish efficacy under natural field conditions.

The virus resistant tomato project in West Africa was one of the best examples ever witnessed of multi-country cooperation among the West African NARS. The NARS from Mali, Ghana, Benin, Togo, Burkina Faso, Niger and Senegal all worked together on a unified effort to identify TYLCV resistant tomato varieties for West Africa. Dr. Molly Jahn from Cornell University used her relationship with most of the tomato seed companies to convince them to share their highly prized hybrid germplasm with the NARS to be tested under West African conditions for TYLCV resistance. Each of the participating NARS partners ran the exact same experiment measuring the same parameters in unison to identify tomato varieties that showed resistant to TYLCV with consumer desirable traits. Several good varieties were identified that worked well across the entire region. The TYLCV was so severe in the country that potyvirus became an insignificant problem in West Africa so ABSPII discontinued efforts to introduce the potyvirus resistance technology into tomato for West Africa.

LESSONS LEARNED AND FUTURE WORK TO BE DONE

The FSB resistant eggplant work facilitated by ABSPII proved to be an effective model of how to achieve impact through public-private and south-south collaboration. However, important lessons have been learned that will influence the direction of future work on crops developed

through biotechnology. The most important of these lessons is the role that governments have in facilitating their commercialization, as shown by the results of Bt eggplant in Bangladesh compared to India. In Bangladesh, seeds of Bt eggplant are being made available at low cost to help eggplant farmers increase eggplant crop yields while reducing input costs for pesticides and labor. In India Bt eggplant has been stalled in the courts since February 2010.

The commercial release of Bt eggplant in Bangladesh is a major accomplishment for all parties involved and a game-changer since this was the first biotech food crop released in a developing country. It also sets the stage for the future in which commercial eggplant farmers will have access to more desirable hybrid eggplant varieties made available by Mahyco and others for a technology fee in Bangladesh, India and by UPLB-IPB in the Philippines, if they are approved in these other countries. All of the socio-economic ex-ante impact studies commissioned by ABSPII indicate the likelihood of strong benefits for stakeholders resulting from FSBR eggplant. Research economists concluded that, based on field trial data, the technology could result in significantly reduced insecticide applications and increased marketable yields as is currently being witnessed in Bangladesh. Bt eggplant has been one of ABSPII's greatest achievements and the entire ABSPII team is proud to be part of this great success. Looking back on the project, it is clear that the introduction of Bt eggplant was due not only to the technology, but even more so by the political will of government officials in Bangladesh. The needed support of the government and the power of anti-biotech groups is clearly a major lesson learned. The best technology can be undermined by the lack of an effective communication strategy.

In India, ABSPII witnessed the power of the anti-GM campaign and gained valuable respect for the need for a much more pro-active grassroots communications effort directed at producers and consumers. We clearly underestimated the impact of the grassroots farmer/consumer misinformation campaign of Greenpeace and other anti-GM-crop NGOs which successfully undermined the commercial approval of Bt eggplant in India. We are hopeful that sound science and the solid socio-economic benefits of adopting FSBR eggplant, along with pressure from the scientific community and farmer groups, will result in FSBR eggplant being released for commercial propagation in India in the near future.

In the Philippines, ABSPII was fortunate to closely partner with the most influential, respected and knowledgeable communication institutions in Southeast Asia. For example, ISAAA, SEARCA and CropLife helped ABSPII host over 100 outreach and communications workshops in the Philippines since 2007. ABSPII worked with these same partners during the first phase of ABSPII and hosted almost as many workshops as during the second phase of the project. We were proactive in emphasizing the role of the public institutions in getting the product developed and approved for commercial propagation in the Philippines. It was important that we properly

communicated that Bt eggplant varieties are public property. This resulted in a real sense of ownership by the public partners. For example in the Philippines, the Bt eggplant is known locally as the "Pinoy GM" because of the local scientific and community support for the product. Many students working on the project were educated at UPLB and received BS, MS and PhD degrees. It should be noted ABSPII provided significant capacity to the satellite universities that worked with us on the confined field trials. The people working for the IBC received on-site experience working with the Bt eggplant technology so there was no misunderstanding or misrepresentation of the efficacy conferred by the product. The relationship that ABSPII established with the IBC will be very helpful for the regulatory efforts and the approval for commercial propagation of Bt eggplant in the Philippines. This is the first "Pinoy GM" to be developed and commercialized in the Philippines and the first time that the entire regulatory system in the Philippines will be directly involved from start to finish. Because the Philippines already propagates GM corn for insect resistance, it is wrongly assumed by most people that the Philippines has a mature regulatory system. They have a system that is functional, but it is far from being perfect. There is definitely a need for significant capacity building in the area of biosafety and environmental risk assessment.

Another lesson learned is that approval of a biotech product is only the first step in what is a longer road for successful full commercialization. For example, in Bangladesh, the successful introduction and full approval for propagation of FSBR eggplant will be a function of BARI producing sufficient quantities of breeder seed of the approved varieties to hand off to the Bangladesh Agricultural Development Corporation (BADC) or other commercial seed producers. Furthermore, there are stewardship responsibilities associated with regulatory approval that need to be supported and administered by BARI or some other organization in Bangladesh capable of providing specific training for extension specialists and research scientists. This includes monitoring for changes in susceptibility to the Cry1Ac protein in the field. Training of individuals and carrying out these activities is essential but requires commitment by government agencies or companies and could be a major challenge.

To ensure the durability of FSBR eggplant, development of an eggplant expressing two insecticidal traits to increase durability of the technology will eventually enter the BARI pipeline of GM products. This introduction will require training people to understand and implement stewardship programs in each country. ABSPII did an admirable job of capacity building in India, but more training needs to be done for successful introduction, adoption and maintenance of the FSBR technology in Bangladesh, India and the Philippines

ABSPII has tried to be transparent, compliant and cooperative in its work but working with various agencies in each government has at times been challenging. In the future, improved

communications will be needed to avoid bumps along the road. A well-functioning advisory board can be key to the success of the project. For the first phase and most of the second phase of ABSPII, we had an active advisory board consisting of outstanding scientists and professionals familiar with agriculture biotechnology and the regulatory environment driving commercialization and public acceptance of the technology. We held annual meetings where we thoroughly reviewed every aspect of each PCP. The board members always provided constructive, well-justified criticism and their advice on the best way forward was appreciated and taken seriously.

Another important exercise required by all US-AID projects is an external review towards the end of the project. This external review should be taken seriously and the project leaders should be active in the selection of the review committee. ABSPII had two external reviews. The first external review team was made up of several professional people with a strong agriculture background and good reputations for being objective and fair. Their review was quite thorough as they visited each partner institution and asked the proper questions. Their report accurately captured our strong and weak points, but in a very understanding and objective way. The team selected to review the second phase of ABSPII was made up of two individuals who we believe were unsuitable for a fair and productive review and we asked they not be included. However, upon USAID's insistence to include these individuals, we elected to move forward with the external review regardless of the unacceptable review team. As expected, the external review report was a hostile and baseless debacle that resulted in a major embarrassment for USAID and Cornell University. The lesson learned from this experience is that a well-qualified and fair review team is essential for USAID and the home institution to gain valuable insight so the project can be improved.

Another valuable lesson learned from ABSPII is the importance of including gender in its activities. Considering the fact that women play a major role in scientific advancement as well as farming and communication, the project embarked on a gender parity approach. Several women were trained in various activities of the entire value chain of the project, including product development, regulatory functions, communication, and extension. As well, women farmers were trained in cultivation practices. Three women scientists form BARI, Bangladesh were trained on different aspects of product stewardship including molecular biology techniques, transformation and molecular characterization for the development of FSBR eggplant and LBR potato in Bangladesh. Capacity building of two scientists from India was conducted on the molecular characterization of transgenic products with a focus on FSBR eggplant and DST rice. One scientist was trained in studies on the socio-economic impact of Bt eggplant cultivation in India.

Capacity building was an integral part of ABSPII and should be a key component of future USAID projects. Capacity building not only included training for scientists in South Asia, various trainings and workshops were conducted for farmers, researchers, policy makers and professional communicators. Over a period of 12 years: 150 women scientists were trained on the technical aspects of transgenic products in South Asia; 200 women participants were trained on cultivation practices and Integrated Resistance Management (IRM); and 550 women communicators were trained on the benefits of the GM technology for communication and information dissemination. These efforts in capacity building will be an enduring legacy of ABPSII in each of its participating countries.