



Agricultural Biotechnology Support Project II, South Asia

Supporting agricultural development through biotechnology

NEWSLETTER

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About ABSPII

The developing world can benefit from advances in biotechnology, but much needs to be done to make bio-engineered products available in forms that farmers can use. The Agricultural Biotechnology Support Project II (ABSPII) believes that farmers and consumers worldwide should have the opportunity to make informed choices about using bio-engineered products. ABSPII focuses on the safe and effective development and commercialization of bio-engineered crops as a complement to traditional and organic agricultural approaches in developing countries. The project helps boost food security, economic growth, nutrition and environmental quality in East and West Africa, Indonesia, India, Bangladesh and Philippines. Funded by the United States Agency for International Development (USAID) and led by Cornell University, ABSPII is a consortium of public and private sector institutions.

The consortium develops innovative, pragmatic solutions, building on the successes of the Agricultural Biotechnology Support Project (ABSP) that was led for a decade by Michigan State University.

In South Asia (India and Bangladesh), ABSPII supports development of expertise in the areas of research, policy development, licensing and outreach to help reduce poverty and hunger through agricultural biotechnology. Current initiatives relate to development of Tobacco Streak Virus Resistant (TSVR) groundnut, Late Blight Resistant (LBR) potato, Fruit and Shoot Borer Resistant (FSBR) eggplant, drought tolerant rice and salinity tolerant rice.

Message from K. Vijayaraghavan

Regional Co-ordinator, ABSPII, South Asia

I am delighted to convey that ABSPII partners have successfully identified potential opportunities for mitigating drought and salinity in rice with initial success established in over expression of Trehalose, a non-reducing disaccharide that confers the salinity and drought tolerant trait in rice. The successful efforts of ICGEB in this regard are commendable.

Drought and salinity are the two factors that would impact the productivity of grains and minor crops significantly in the coming years. Over 70 million hectares of rice growing land is affected worldwide due to drought. More than 100 million hectares of agricultural land has turned uncultivable due to salinity impact and nutrient stress factors worldwide. We do perceive considerable public-private partnership opportunities for development of drought and salinity tolerant trait factors that would help farmers to gain sustainable income from lands that are prone to abiotic stress factors.

ABSPII partners are constantly engaged in sharing knowledge across the communities of stakeholders. A recent interview by Dr. P. Balasubramanian of Tamil Nadu Agricultural University highlights the process adopted for the development of fruit and shoot borer resistant eggplant. The gains that this product could provide to farm communities and consumers are of potential interest to a wide audience of stakeholders. I invite you to log on to <http://www.absp2.net> to view the interview.

This issue focuses on developments taking place in Asia for new trait development and trait integration. Asia will emerge as the world's primary vegetable growing region and in this context the developments taking place in countries such as India, China, the Philippines, Taiwan, Malaysia, Vietnam and Indonesia would have greater impact. The Asian countries are ramping up their investment in research for crop yield improvement, trait development and trait integration and these investments will begin to impact the farmers and consumers in the next ten years or so.

FEATURE

Transgene RB functions in Multiple Potato Backgrounds for Agriculturally Meaningful Levels of FLB Resistance*

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Despite progress made during more than a hundred years of breeding effort, commercially prominent potato cultivars carrying durable resistance to late blight remain elusive. The RB gene from the wild potato *Solanum bulbocastanum* imparts foliar resistance against a broad array of races of *Phytophthora infestans*, the causal agent of late blight. Plants carrying the RB gene display a partial resistance phenotype characterized by reduced, but not entirely eliminated, pathogen growth. A lack of race specificity and a partial resistance phenotype are qualities that are thought to favor disease resistance gene durability. Thus, although durability can only be demonstrated after years of large-scale deployment, researchers remain optimistic that RB might be durable in the agricultural setting. The recent cloning of RB (Song et al., 2003) offers the potential to now use transgenic approaches to enhance commercially prominent potato cultivars, rendering them disease resistant. Because a transformed line will remain morphologically equivalent to the untransformed cultivar, save the addition of late blight resistance, transgenic approaches circumvent disruption of favorable gene combinations that result from traditional breeding approaches (reviewed in Bradeen et al., 2008). In collaboration with Sandra Austin-Phillips (University of Wisconsin), we have generated 57 transgenic potato lines, each carrying one or more copies of the RB transgene under the control of the native *S. bulbocastanum* promoter. Multiple independent transformed lines have been generated for each of four important potato cultivars grown in the US: 'Russet Burbank', 'Superior', 'Dark Red Norland', and 'Katahdin'. These cultivars represent processing, chipping, and fresh market types and encompass a wide genetic background. We challenged each

of these lines with *P. infestans* US8 in a two year, replicated field trial conducted at the University of Minnesota. Additionally, we utilized a previously developed quantitative RT-PCR assay (Millett and Bradeen, 2007), to measure RB transcript levels in unchallenged foliage. Here I present results of our study and discuss their implications for transgenic deployment of RB.

Field Performance of RB Transgenics

Under field testing conditions at the University of Minnesota, RB transgenics, on the whole, proved to be substantially more resistant to *P. infestans* US8 than their untransformed counterparts.



Figure 1: The RB transgene imparts foliar late blight resistance. A late blight nursery at the University of Minnesota 28 days after inoculation with *P. infestans* US8. Rows of transgenic plants (green) are sandwiched between rows of non-transgenic potato 'Norchip' (brown, dead from late blight disease). The RB transgene protected lines of four different potato cultivars in the absence of fungicides, demonstrating that the gene is effective in multiple genetic backgrounds. Photograph was taken in 2005.

Resistance data collected in 2005 and 2006 and were strongly correlated ($R^2 = 0.68$, $p < 0.01$; Figure 2), indicating that the transgenic lines performed similarly in both years. This suggests that RB-mediated resistance might remain stable across environments. Based on visual ratings of disease development, we have defined four phenotypic classes: "susceptible", "moderately susceptible", "moderately resistant", and "resistant". As shown in Figure 2, the tested transgenic lines represent a continuum of phenotypes from resistant to susceptible, consistent with the partial resistance nature of this gene. Importantly, 39 of the 57 (68.4%) lines tested displayed a "resistant" or "moderately resistant" phenotype. These lines are the most promising for direct agricultural application. Under average growing conditions in Minnesota, we anticipate that lines ranked in our study as "resistant" or "moderately resistant" will yield well, even in years of severe late blight pressure, with limited fungicide application. More detailed analysis is required before

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recommendations for fungicide applications on these materials can be provided. Significantly, "moderately resistant" transgenic lines were identified for each of the four tested potato cultivars and "resistant" transgenic lines were identified for all cultivars except 'Superior'. This indicates that RB is effective in a broad array of genetic backgrounds. Nevertheless, we anticipate that background will have some effect on RB function. If this proves to be the case, researchers may need to screen a larger number of independent transformants for some cultivars to identify lines with acceptable levels of disease resistance. At present, it is not possible to predict in which genetic backgrounds RB will have the strongest effect.

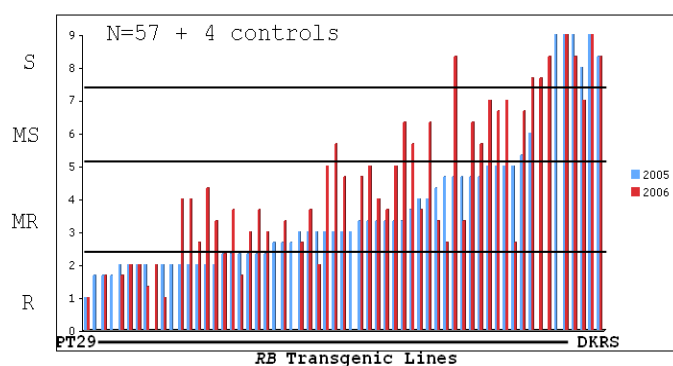


Figure 2: RB transgenic lines represent a range of phenotypes; perform similarly across years. Fifty-seven RB transgenic lines, untransformed controls (D – ‘Dark Red Norland’; K – ‘Katahdin’; R – ‘Russet Burbank’; S – ‘Superior’), and *S. bulbocastanum* genotypes PT29 were visually rated for foliar late blight resistance in replicated field trials conducted in 2005 and 2006 at the University of Minnesota. Plants were challenged with *P. infestans* US8 and evaluated 28 days post inoculation using a 1 (no disease; fully resistant) to 9 (fully diseased; dead) scale (y axis). Resistance categories (S – susceptible; MS – moderately susceptible; MR – moderately resistant; R – resistant) were defined. Blue bars represent data collected in 2005. Red bars represent data collected in 2006. Data from 2005 and 2006 were highly correlated ($R^2 = 0.68$, $p < 0.01$), suggesting RB will perform similarly in different environments. Importantly, RB transgenic lines display a range of phenotypes from susceptible to resistant. Thirty-nine of the 57 (68.4%) lines tested were classified as resistant or moderately resistant. These represent lines best adapted for production agriculture.

Analysis of RB Transcript Levels

Plant disease resistance proteins are thought to play the roles of cellular sentinels and switches (Fluhr, 2001). As sentinels, it is the role of the plant disease resistance protein to directly or indirectly detect the presence of a pathogen. Once a pathogen has been detected, the plant disease resistance protein triggers downstream cellular responses that result in the phenotype we perceive as resistance. Because the plant disease resistance protein must be present in the plant cell before the pathogen attacks, disease resistance genes are generally thought to be transcribed at low levels at all times in the plant cell. Once

the pathogen attacks, some, but not all, plant disease resistance genes are transcribed at a higher level.

Using our RB transgene-specific RT-PCR assay (Millett and Bradeen, 2007), we surveyed each of our transgenic lines to quantify RB transcript levels. In this experiment, RNA was extracted from field-grown plants immediately prior to inoculation with *P. infestans* US8. Thus, our data reflect the level of basal transcript levels present in each line, irrespective of pathogen attack. We found that, consistent with reports for other plant disease resistance genes, the RB transcript accumulates in the cell at low levels, even in the absence of the pathogen. Looking across our transgenic lines, substantial variation in RB transcript levels was noted. Importantly, a clear trend of increasing disease resistance with increasing RB transcript levels was evident.

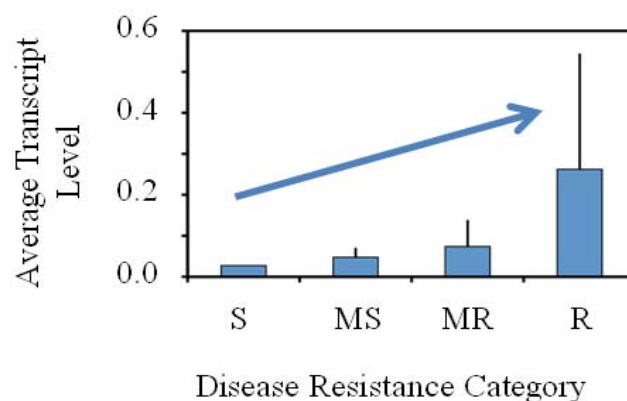


Figure 3: Higher RB transcript levels correspond to improved foliar late blight resistance. Foliar late blight disease resistance categories (x axis; S – susceptible; MS – moderately susceptible; MR – moderately resistant; R – resistant) of +RB transgenic potato lines were plotted against average normalized RB transcript levels (y axis), revealing a trend of increasing disease resistance with increasing transcript levels. For researchers, this suggests RB transcript levels can be used to “pre-screen” transformed plants prior to phenotype characterization; plants possessing high levels of the RB transcript are more likely to be late blight resistant. Alternatively, artificially increasing RB transcript levels may yield improved disease resistance.

This finding suggests that RB steady state transcription is one means by which the plant balances effective resistance against the late blight pathogen on one hand, and metabolic costs associated with resistance responses on the other hand.

For researchers, the correlation between transcript levels and disease resistance has two important implications. First, where opportunity to maintain and test large numbers of transgenic lines is a limitation, quantitative RT-PCR can be utilized to identify individual lines with comparatively high RB transcript levels. These lines, which are more likely to be late blight resistance, can then be

advanced to phenotypic assays. Conversely, artificial manipulation of RB transcript levels could be a mechanism for enhancing resistance. In this study, the RB transgene was driven by the native *S. bulbocastanum* promoter. The use of this promoter in combination with enhancer elements or the use of alternative promoters might yield higher transcript levels. While it is likely that there is an upward limit of effectiveness of increasing RB transcript levels on disease resistance, our current data suggest we have not yet reached this limit.

Implications for Durability and Future Research.

This study has taught us much about the behavior of the RB transgene under field conditions and has suggested means of more effectively using it to achieve agriculturally meaningful levels of resistance. Sadly, history has taught us that *P. infestans* is a formidable foe. There have been other foliar late blight resistance genes that have been hailed as the final answer to the disease. Each time, however, the pathogen population has adapted and shifted, rendering once-promising disease resistance genes ineffective. RB displays characteristics that are thought to favor long-term durability including a lack of pathogen race specificity and a plant phenotype of delayed but not eliminated disease development. Still, researchers will be wise to develop deployment strategies that reduce the likelihood the pathogen will adapt to overcome RB-mediated resistance. The recent cloning of additional late blight resistance genes from *S. bulbocastanum* and other wild potato species (e.g., Ballvora et al., 2002; Huang et al., 2005; Vossen et al., 2005) now offers the potential to pyramid resistance genes, providing a more complex resistance response to which a pathogen is less likely to adapt. Integration of RB into a no- or reduced-fungicide production environment will be aided by understanding the basic biological function of the gene. Towards this goal, other lines of research in our laboratory will explore the potential to use RB for tuber late blight control and will yield an understanding of how plant age impacts RB function.

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Setting the Record Straight: A Rebuttal to an Erroneous Analysis on Transgenic Insecticidal Crops and Natural Enemies

An article by Lövei et al. "Transgenic insecticidal crops and natural enemies: a detailed review of laboratory studies" published in *Environmental Entomology* 38(2): 293-306, 2009 purports that insect-protected crops based on the Cry proteins of *Bacillus thuringiensis* may have substantial negative impacts on non-target organisms. A group of experts in this area strongly disagreed with this April, 2009 publication and felt that a rapid response was required but, because of production schedules of this bi-monthly journal, it could not accommodate a rapid rebuttal. Thus, A. M. Shelton and 14 colleagues published their Letter to the Editor in *Transgenic Research* ("Setting the Record Straight: A Rebuttal to an Erroneous Analysis on Transgenic Insecticidal Crops and Natural Enemies").

Among the many concerns Shelton and colleagues describe in their rebuttal are the inappropriate and unsound methods for risk assessment that led Lövei et al. to reach conclusions that are in conflict with those of several comprehensive reviews and meta-analyses. Shelton summarized the concerns of the 15 authors by stating, "The Lövei et al. article advocates inappropriate summarization and statistical methods, a negatively biased

and incorrect interpretation of the published data on non-target effects, and fails to place any putative effect into a meaningful ecological context." What was also troubling to this international group of 15 experts is the potential for the Lövei et al. article to be accepted at face value and impact some regulatory agencies.

Their rebuttal can be accessed using the following link: <http://www.springerlink.com/content/q7hk642137241733>

The article is open access and freely available to all and is published as DOI: 10.1007/s11248-009-9260-5.

EVENTS

ABSPH Director Visits LBR Potato Field Trial Site in Bangladesh

Dr. Frank Shotkoski, the Director of ABSPH visited the two LBR potato field trial sites at Joydebpur and Debigonj in late February 2009. He was accompanied by Dr. G. P. Das, Country Coordinator for the ABSPH South Asia project in Bangladesh.



Figure 1: ABSPH Director Dr. Frank Shotkoski with BARI officials at the LBR potato field trial site at Joydebpur

Confined field trials were set up by BARI at Joydebpur and Debigonj with transgenic hybrid and transformed clones of Cardinal and Diamant. The objective of the confined field trial was to observe the performance of such lines under late blight infection in the field. The non transgenic plants have been damaged by the late blight disease, while transgenic lines have shown significant resistance towards late blight disease. It looks like that the transgenic lines are in a

position to drastically reduce the infection of late blight disease. The trials have been accomplished and data and results indicate that the transgenic hybrid clones were resistant to late blight, good in plant vigor and high in tuber yield compared to non transgenic plants.

COTS Launched at UAS, Dharwad

Understanding the high value that an effective Management Information System (MIS) can offer for the seed industry, Sathguru Management Consultants, Hyderabad has designed and developed a unique and focused software solution called the Computerized Operation Tracing System or COTS. COTS streamlines the complete value chain in organizations involved in seed production, beginning with indenting to the distribution of seeds.



Prof. J. H. Kulkarni Vice-Chancellor of UAS, Dharwad launches the COTS package at the seed unit while Prof. P. M. Salimath Director of Research and Prof. L. Krishna Naik Director of Seeds look on

As part of the ABSPH Bt eggplant seed delivery initiative, the ABSPH team has installed COTS and customized the package based on a public institution seed delivery system. On 4 May, 2009 Dr. J. H. Kulkarni, the Vice-Chancellor of the University of Agricultural Sciences, Dharwad launched the COTS package at the university's Seed Unit. The official launch was followed by a speech by Dr. L. Krishna Naik, Director of Seeds at the university, who emphasized the value of COTS and the vital role it will play in enhancing the public sector's seed supply chain. He also added that COTS is a product evolved from the amalgamation of ideas of skilled professionals who have dedicated themselves for years to produce quality seeds to

improve the quality of life for resource constrained farmers and, in turn, bring greater benefits to society.

Representatives from the COTS development team and ABSPII, South Asia demonstrated the functions available in the COTS package and explained how it could help generate reports which would help management make informed decisions.

Prof. J. H. Kulkarni, addressed the gathering and emphasized the importance of using COTS on an everyday basis. He also stressed the productivity and the accuracy of the information/reports that COTS creates. He also added that this software package will create significant impact on transgenic Bt eggplant seed production and distribution to resource constrained farmers in southern India.

Capacity building program for COTS package at UAS, Dharwad



Prof. Kulkarni, Vice-Chancellor of UAS Dharwad launches the capacity building program for the COTS package

The ABSPII team organized a COTS user training program for the seed production and distribution team at UAS, Dharwad on 21 and 22 May 2009. Thirty participants attended the training program, including members of the main seed unit team and research network station in Karnataka. Mr. Jeevan, a key member of the COTS development team, conducted the training session for various components of the COTS package. This involves a seed demand forecasting module, seed production module, seed processing, delivery module and the management information system. The trainer responded to queries raised by the participants, on the various functionalities of

the COTS package. The package is expected to enhance the Bt eggplant seed supply chain in southern India.

REPORT

Bt. Brinjal: To Goa for the Answers

Bt brinjal will soon be the first transgenic vegetable crop in India. This will provide over 14 lakh farmers in the country with a safe and cost-effective pest management solution against the dreaded fruit and shoot borer (FSB) pest that accounts for damage to as much as 70 percent of the average brinjal crop and a cumulative financial loss to the country to the tune of INR 1,000 crore per annum.



Prof. Chandrasekhar of UAS Dharwad explains the FSB Brinjal to press delegates at the trial site in Goa

The South Asia Regional Coordination Office of the Agricultural Biotechnology Support Project II (ABSP II) played a host to representatives from leading Indian news and business publications who had the opportunity to interact with the scientists involved in the development of Bt Brinjal in India. This press meet was held on 13 April, 2009 in Goa. The press meet was followed by a visit to a trial site where the journalists were able to witness the Bt technology in action. The developing scientists represented University of Agricultural Sciences (UAS), Dharwad, Tamil Nadu Agricultural University (TNAU), Coimbatore, and Mahyco.

The Bt brinjal varieties were developed to effectively counter the ravages of the Fruit and Shoot Borer (FSB), a larva that accounts for damage to as much as 70 percent of the average brinjal crop. The introduction of this technol-

ogy ultimately translates to greater returns for the farmer who will be able to spray less of the expensive pesticides typically used to destroy these pests. The Bt brinjal will have a higher yield of healthy fruits undamaged by the FSB which will also contain less residue from the toxic chemicals in the pesticides that have until now been sprayed to protect the plant from these pests.

Mr. Vijayaraghavan, the South Asia Coordinator of the ABSPII, along with Dr. Balasubramanian from TNAU, Dr. Bharat Char from Mahyco and Dr. P. M Salimath from UAS Dharwad apprised the participating journalists on ABSPII and its aim in harnessing public sector potential and apply proven technologies to augment food security and environmental safety and upliftment of living conditions of resource constrained farmers.

Information on Bt brinjal product development and details of the socioeconomic studies that were undertaken were shared with the press. A short video film which was aired featuring Prof P. Balasubramanian answering questions on the Bt technology, its safety and efficacy in a food crop such as Brinjal was much appreciated. This paved way to an open discussion on various questions relating to the safety and consumer acceptance of Bt Brinjal among the press and the scientists. The scientists addressed questions from the press and were able to quell the fears, doubts and misconceptions on transgenic technologies predominantly due to lack of awareness. The safety of the technology was elaborated by discussing the stringent tests that are recommended by the Indian government to ensure the safety of the developed product.

During the visit to MLT trial site at Margoa, the press witnessed first hand, the technology at work. Bt. and non Bt. crops grown side by side displayed marked differences in FSB infestation.

ABSPII Shines Again on AUTM

For the second year in a row an ABSPII South Asia project was featured in The Better World Report of the Association of University Technology Managers (AUTM). This year, the development of potato varieties that are resistant to late blight has been listed as one of hundred "Innovations from Academic Research that Positively Impact Global Health."

Late blight, a plant disease caused by a fungal pathogen, was responsible for the Irish Potato Famine of the mid-1800s. More recently, it was responsible for losses of as much as 70 percent of India's 1.28 million hectares of potato crops in 2007. In Bangladesh, the disease has attacked 50 percent of potato crops. Farmers who grow the

vegetable in these countries can barely afford the high price of the pesticides, herbicides and fungicides required to control the disease. To add to the crisis, the disease has developed a resistance to the chemicals. Farmers thus tend to overspray thereby posing health and environmental risks to the region.



Prof. K. V. Raman of Cornell University at the LBR Potato trial site at the Central Potato Research Institute, Shimla

A gene found in *Solanum bulbocastanum*, a wild relative of the potato, was found to make it resistant to late blight infection. After limited success in cross breeding the resistant variety with common cash crop potatoes, researchers at the University of Wisconsin- Madison were able to isolate the resistant gene, Rb, and use genetic engineering to insert it into popular U.S. potato varieties.

Sathguru Management Consultants in India coordinated with the university to use this gene technology pro bono and develop resistant cultivators in India and Bangladesh. A global consortium under the United States Agency for International Development's Agricultural Biotechnology Support Project II (ABSPII) was formed for this project, including the University of Wisconsin-Madison, Sathguru, Cornell University, Central Potato Research Institute in India and Bangladesh Agriculture Research Institute.

With funding from ABSPII, governments in India and Bangladesh, Cornell, Sathguru and the University of Wisconsin-Madison, researchers have introduced the Rb gene to popular local potato varieties. In India, Kufri Jyothi and Kufri Bahar varieties have been modified and tested, and in Bangladesh, Diamant and Cardinal varieties are being assessed. Successful trials have led researchers to believe that soon new products will be available to farmers and become an integral part of pest management systems for late blight.

FORTHCOMING EVENTS

Seed Industry Program 2009

Cornell-in-India in association with Sathguru will be offering a four-day residential course on Seed development and delivery for the private and public sector from 26-30 October 2009. Faculty from Cornell University and globally experienced senior consultants from Sathguru will lead the sessions which will be held at the Marriott, Hyderabad.

The program will focus on enhancing the ability of the participants to understand emerging issues and develop strategic solutions to augment business through deeper understanding of the business growth drivers, research management, technology access through licensing, regulatory, market access factors and business restructuring needs of the seed sector.

Past participants for the program have included representatives from private and public enterprises and academia. Directors, Crop Managers, Heads of Marketing, Heads of Research, Senior Managers, Product Managers, Heads of Product Development, Heads of Regulations, Heads of Operations, Professors and Scientists will ideally gain through participation in this program.

For more information on the program, please visit:
<http://www.sathguru.com/seeds>
or email your queries to:
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