SOUTH ASIA
BIOSAFETY PROGRAM

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NEWSLETTER

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SABP

The South Asia Biosafety Program (SABP) is an international developmental program initiated with support from the United States Agency for International Development (USAID). The program is implemented in India and Bangladesh and aims to work with the local governments to facilitate implementation of transparent, efficient and responsive regulatory frameworks that ensure the safety of new foods and feeds, and protect the environment.

SABP is working with its in-country partners to:

- Identify and respond to technical training needs for food, feed and environmental safety assessment.
- Develop a sustainable network of trained, authoritative local experts to communicate both the benefits and the concerns associated with new agricultural biotechnologies to farmers and other stakeholder groups.
- Raise the profile of biotechnology and biosafety on the policy agenda within India and address policy issues within the overall context of economic development, international trade, environmental safety and sustainability.

TRANSGENIC POTATO FOR ECO-FRIENDLY MANAGEMENT OF LATE BLIGHT IN INDIA

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Late blight caused by the fungus-like brown algae *Phytophthora infestans* is the most dreaded disease in potatoes. It causes a worlwide annual loss of approximately US$ 3.0 billion. In India, management of late blight through host resistance would save around US$ 250-350 million annually. Moreover, heavy doses of fungicides (Metalaxyl and Mancozeb) are sprayed on potato crops to save them from this devastating disease thus creating a serious environmental hazard. Development of potato cultivars with durable late blight resistance is, therefore, a major research and development activity of many potato-growing nations.

Breeding for late blight resistance began nearly one hundred years ago with the introgression of resistance from *Solanum demissum*, a wild hexaploid species indigenous to Mexico. Resistance in *S. demissum* is determined by dominant *R* genes inducing a hypersensitive (HR) response upon infection with specific races of *P. infestans*. This type of resistance, often called vertical resistance, however, breaks down quickly due to emergence of new pathogen races. Therefore, thrust in late blight breeding has now shifted to deployment of multi-gene, horizontal resistance. Identification of candidate genes responsible for horizontal resistance and their pyramiding is a formidable task that can't be achieved in the foreseeable future. Recently, a major gene (*RB*), behaving like non-host resistance and effective against all known races of *P. infestans*, has been mapped and cloned by two independent groups in the United States and in the

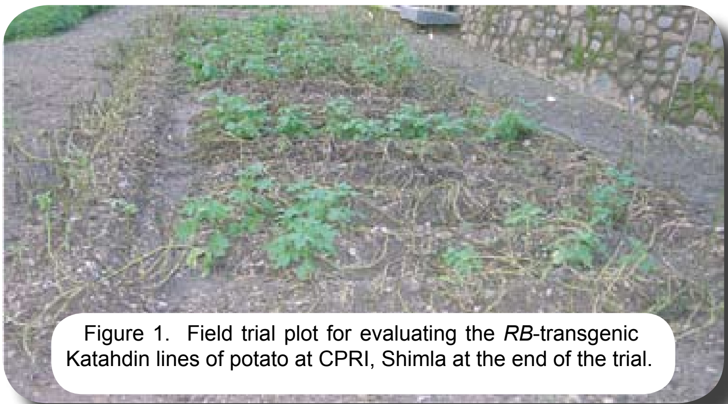

Figure 1. Field trial plot for evaluating the *RB*-transgenic Katahdin lines of potato at CPRI, Shimla at the end of the trial.

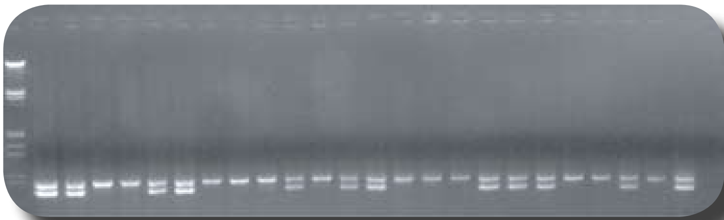

Figure 2. Multiplex PCR of F_1 hybrid samples obtained from the cross Kufri Jyoti X SP951 using RB-specific MAMA primers (fragment size 690 bp) and the endogenous control RNA polymerase II gene (fragment size 862 bp) for confirmation of *RB* gene.

Figure 3. Late blight reaction on a hybrid (KJSP951-105, centre) of Kufri Jyoti along with the maternal parent Kufri Jyoti (left) and the paternal parent SP951.

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CALENDAR OF EVENTS

Event	Organization	Date	Place
INDIA			
A practical training course on "Genomics, transformation and molecular marker tools for crop improvement"	Department of Biotechnology & Molecular Biology, CCS Haryana Agricultural University	May 19 - June 9, 2009	CCS HAU, Hisar
A practical training course on "Techniques in plant tissue culture, genetic engineering and molecular biology"	Department of Biotechnology & Molecular Biology, CCS Haryana Agricultural University	May 19 - June 9, 2009	CCS HAU, Hisar
Bangalore BIO 2009	Department of IT and Biotechnology, Government of Karnataka and the Vision Group on Biotechnology	June 18 - 20, 2009	Bangalore
Applications of Biotechnology and Its Regulations	The Energy and Resources Institute (TERI)	August 4 - 21, 2009	New Delhi
National Seminar on Nutritional Strategies for Improving Quality of Life	Department of Foods and Nutrition, College of Home Science, G.B. Pant University of Agriculture and Technology (GBPUAT)	September 11 - 12, 2009	GBPUAT, Pantnagar, Uttarakhand
INTERNATIONAL			
Theoretical and practical course 'Developments in Biosciences for Enhanced Food and Environmental Biosafety'	Department of Molecular Biology and Biotechnology, Faculty of Science, University of Dar es Salaam, Dar es Salaam, Tanzania	August 18 to 30, 2009	Department of Molecular Biology and Biotechnology, Faculty of Science, University of Dar es Salaam
An International Short Course in Agricultural Biotechnology	Institute of International Agriculture, Michigan State University	September 13 - 25, 2009	Michigan State University
ABIC 2009: Agricultural Biotechnology for Better Living and a Clean Environment	National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), Ministry of Science and Technology (MOST) and ABIC Foundation	September 22 - 25, 2009	Queen Sirikit National Convention Center, Bangkok, Thailand
Biosafety workshop on 'Theoretical Approaches and Their Practical Application in the Risk Assessment for the Deliberate Release of Genetically Modified Plants'	Wendy Craig (Biosafety Unit, ICGEB, Trieste, Italy)	October 12 - 16, 2009	ICGEB Conference and Meetings, Padriciano 99, I-34012 Trieste, Italy

Potato - *continued from page 1*

Netherlands. The *RB* gene has withstood the onslaught of *P. infestans* for more than five decades. Transgenic clones of the potato cultivar Katahdin encoding this gene showed late blight resistance at Toluca valley, the center of origin of *P. inefstans*. The Agricultural Biotechnology Support Project-II (ABSPII) initiated a programme to popularize the use of *RB* gene in South and South-east Asia. After getting permission from the Review Committee on Genetic Modification (RCGM), two transgenic lines (SP 904 and SP 951) of the potato cultivar Katahdin were imported from the University of Wisconsin and evaluated under limited field trial at Shimla during 2006. Both the lines showed a remarkable level of field resistance to Indian isolates of *P. infestans* (Figure 1). A collaborative project between ICAR and ABSPII is now operating at CPRI, Shimla to use the *RB* gene for developing varieties with durable late blight resistance.

Two parallel approaches are being followed in India to utilize the *RB* gene. In one strategy, the well adapted Indian potato cultivars like Kufri Jyoti and Kufri Bahar have been crossed with the *RB*-transgenic Katahdin as male parent. The F_1 hybrids were checked for the presence of the *RB* gene by using a gene-specific SCAR marker (Figure 2) and the positive clones were screened for late blight resistance in a controlled environment chamber. The resistant clones were multiplied in a transgenic containment facility for further selection on the basis of tuber shape, size, colour, uniformity, eye depth, *etc*. The selected clones were advanced to the next clonal generation for ultimate development of new late blight resistant varieties using a conventional breeding approach. In the conventional breeding approach, the F_1 clones obtained from a cross are checked for tuber characteristics, storage behavior, yield and clonal stability for eight to nine consecutive generations before selecting a potato variety. Promising clones carrying the *RB* gene and showing a good level of late blight resistance, tuber characteristics and yield have been identified and so far advanced to a third clonal generation. The clones obtained from the cross of Kufri Jyoti (Figure 3) showed better late blight field resistance than those obtained from Kufri Bahar. The selected clones are to be evaluated under field conditions for identification of promising ones with a good level of field resistance to late blight under epiphytotic conditions. Permission of RCGM has been sought for a confined field trial with the selected clones.

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CREAM OF THE (WEB) CROP

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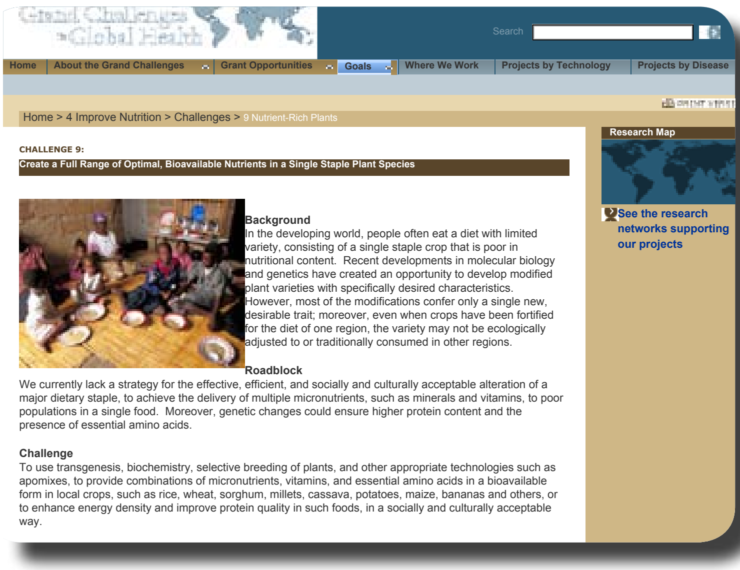
THIS MONTH'S PICK:

Grand Challenges in Global Health

http://www.grandchallenges.org

The Grand Challenges in Global Health initiative was established in 2003 by The Bill & Melinda Gates Foundation with the objective of improving health in the developing world. The seven long-term goals of the programme are to improve vaccines, to create new vaccines, to control insect vectors, to improve nutrition, to limit drug resistance, to cure infection and to measure health status. These goals are to be reached by focusing on 14 Grand Challenges. To see a full list of all the Challenges go to http://www.grandchallenges.org/Pages/BrowseByGoal.aspx.

Falling under the goal "to improve nutrition", Challenge Number 9 is to use transgenesis, biochemistry, selective breeding of plants, and other appropriate technologies such as apomixes, to provide combinations of micronutrients, vitamins, and essential amino acids in a bioavailable form in local crops, such as rice, wheat, sorghum, millets, cassava, potatoes, maize, bananas and others, or to enhance energy density and improve protein quality in such foods, in a socially and culturally acceptable way.



Challenge Number 9's intended benefits are the reduction of morbidity and mortality from malnutrition and the amelioration of related susceptibility to infections and other diseases. Its priority is targeting deficiencies in iron, zinc, selenium, vitamins A and E and protein.

Following are the projects that are being conducted to achieve Challenge Number 9's main aims:

Optimisation of Bioavailable Nutrients in Transgenic Bananas

Project members are attempting to genetically modify bananas raised in Uganda so that their content of vitamin A, vitamin E, and iron is equal to or exceeds the required daily allowance. The team is continuing to research the development of "high efficiency" banana transformation systems. Their work is focused on two areas, (1) the isolation of plant or plant-derived anti-apoptosis genes, and (2) the development of systems to express anti-apoptosis genes only at the time of transformation. More information about this project can be found at http://www.grandchallenges.org/ImproveNutrition/Challenges/NutrientRichPlants/Pages/Bananas.aspx.

Engineering Rice for High Beta Carotene, Vitamin E and Enhanced Fe and Zn Bioavailability

Project members are developing new varieties of rice with increased levels or bioavailability of pro-vitamin A, vitamin E, iron, and zinc as well as improved protein quality and content. As their platform, researchers are using Golden Rice, which has been genetically engineered to produce and accumulate pro-vitamin A in the grain, and are working with novel transgene-based technologies to enhance the availability of the target nutrients. The project plans to incorporate the new rice lines as well as Golden Rice into ongoing breeding and seed delivery programs for developing countries, and to make the products freely available to low-income farmers in the developing world. More information about this project can be found at http://www.grandchallenges.org/ImproveNutrition/Challenges/NutrientRichPlants/Pages/Rice.aspx.

Nutritionally Enhanced Sorghum for the Arid and Semi Arid Tropical Areas of Africa

Project members are working to develop new varieties of sorghum that are easier to digest and contain higher levels of vitamins A and E, iron, zinc, and the essential amino acids lysine, threonine, and tryptophan. The project team has focused on developing new sorghum lines and producing seeds while at the same time addressing regulatory issues and public acceptance. They are also developing plans to produce sorghum targeted to specific locations and conditions. Project scientists are working to develop plants and seeds of the second generation of ABS. More information about this project can be found at http://www.grandchallenges.org/ImproveNutrition/Challenges/NutrientRichPlants/Pages/Sorghum.aspx.

Improving Cassava for Nutrition, Health, and Sustainable Development

Project members are working to create nutritious cassava for sub-Saharan Africa. Team members are screening additional transgenic plants and expect that complimentary genetic strategies currently underway will soon yield plants that achieve their targeted levels of iron, zinc, and protein. More information about this project can be found at http://www.grandchallenges.org/ImproveNutrition/Challenges/NutrientRichPlants/Pages/Cassava.aspx.

Potato - *continued from page 2*

In the second approach, the *RB* gene has been transferred into the commercial Indian potato cultivar Kufri Jyoti by *Agrobacterium*-mediated genetic transformation. The transformation and regeneration protocol standardized earlier in the institute by using internodal stem explants of micropropagated plantlets was used to transform the popular cultivar Kufri Jyoti. A total of 86 shoots were regenerated out of which 60 developed roots under kanamycin selection. The positive lines were checked for the presence of the *RB* gene by using three SCAR markers distributed at both the ends and the middle of the gene (about 8.6 kbp long). Though most of the putative transgenic lines were negative for integration of the full length *RB* gene (no amplification of all three fragments), eight independent events have been identified that were positive for the entire gene. Those eight positive transgenic lines of Kufri Jyoti were screened in a controlled environment chamber. All eight transgenic lines showed better resistance than those of non-transgenic Kufri Jyoti. Late blight resistance of the clone KJRBE-58 after screening in a controlled environment chamber is shown in the Figure 4. Field performance of the selected *RB*-transgenic lines has not been evaluated yet.

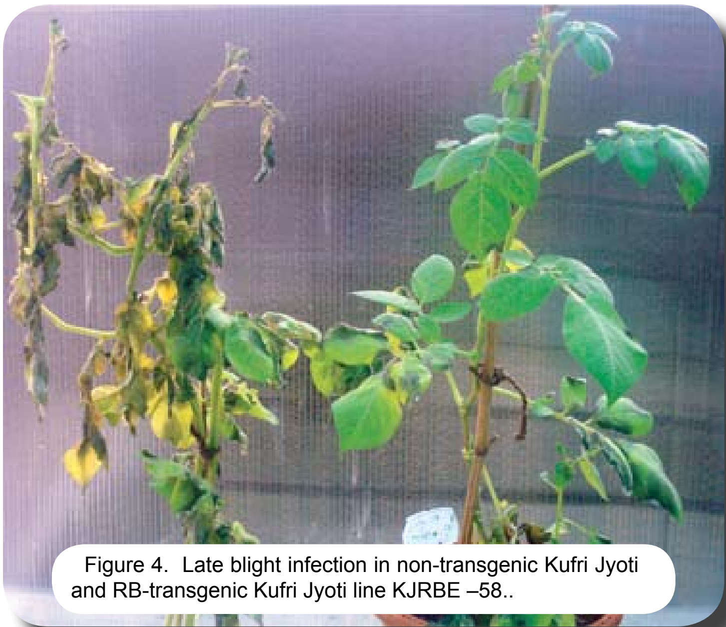


Figure 4. Late blight infection in non-transgenic Kufri Jyoti and RB-transgenic Kufri Jyoti line KJRBE –58..

Development of *RB*-transgenic Kufri Jyoti with durable late blight resistance will be a boon for the resource poor farmers of India. Kufri Jyoti is a very popular variety occupying about 40 per cent of the total potato growing area in the country. The variety was initially released as a late blight resistant cultivar, but its resistance broke down during the 1980s making it susceptible to the disease. Farmers, however, are reluctant to discontinue this variety largely because of its unique adaptability to most of the agro-climatic zones of India. The *RB* gene, therefore, can rescue a popular variety of potato whose blight resistance has been compromised.

We welcome reader comments or suggestions.
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FROM THE NEWS

This recently published article may be of interest to readers of the SABP newsletter.

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

Transgenic Research, June 2009, Volume 18, Number 3, pp 317-322.

Anthony M. Shelton, Steven E. Naranjo, Jörg Romeis, Richard L. Hellmich, Jeffrey D. Wolt, Brian A. Federici, Ramon Albajes, Franz Bigler, Elisabeth P.J. Burgess, Galen P. Dively, Angharad M.R. Gatehouse, Louise A. Malone, Richard Roush, Mark Sears and Frantisek Sehnal

As scientists involved in risk assessment of transgenic insecticidal plants, we are greatly concerned about the publication by Lövei *et al*. (2009) implying that insect-protected crops based on the Cry proteins of *Bacillus thuringiensis* may have substantial negative impacts on non-target organisms. We believe that Lövei *et al*. (2009) use inappropriate and unsound methods for risk assessment that have led them to reach conclusions that are in conflict with those of several recent comprehensive reviews and meta-analyses (*e.g.*, O'Callaghan *et al*., 2005; Romeis *et al*., 2006; Marvier *et al*., 2007; Wolfenbarger *et al*., 2008; Naranjo, 2009). Lövei *et al*. (2009) base their findings on an analysis of 55 laboratory studies of Cry proteins and 27 studies of proteinase inhibitors (PIs; including lectins) that were published through mid-2007 and conclude that these proteins "often have non-neutral effects on natural enemies". They further conclude that "parasitoids were more susceptible than predators to the effects of both (toxins)" and that "conclusions that *Bt*...gene products have no harm to natural enemies are currently overgeneralized and premature". We are deeply concerned about the inappropriate methods used in their paper, the lack of ecological context, and the authors' advocacy of how laboratory studies on non-target arthropods should be conducted and interpreted. Essentially, the authors have conducted a data-mining exercise without prior elaboration of a risk hypothesis framework (Romeis *et al*., 2008) that can provide context to their findings and interpretations. Therefore, we believe it is very important that readers consider the following points as they read Lövei *et al*. (2009).

See the full article at http://www.springerlink.com/content/q7hk642137241733/?p=b415da828f74430187504fa36645e787&pi=0.

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