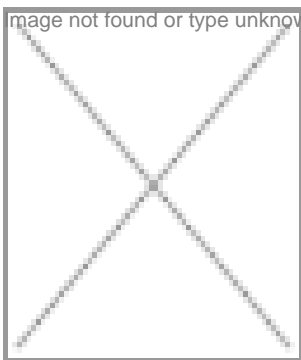


Tireless brains behind Bt brinjal

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Chief technical officer and joint director of research, Mahyco

Zehr has been the brain behind the development of Bt brinjal at Mahyco.

Dr Zehr is the joint director of Research at Mahyco, India. She is responsible for research on plant biotechnology, technology transfer to farmers, utilization of new technologies and tools, including improving the quality and productivity of seeds and agriculture.

Insect-tolerant or Bt brinjal expressing the *Bacillus thuringiensis* cry1Ac gene has been developed to reduce FSB pest-related damage in the brinjal crop, and increase farmers' profitability by way of increased marketable yield and protect the environment pesticides overuse. Ex-ante studies have indicated that deploying Bt brinjal would bring down the costs of cultivation of brinjal, as the contribution of chemical pesticides to brinjal cultivation costs is significant, and highest amongst vegetables in India.

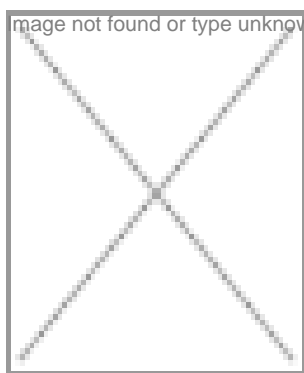
Dr Zehr says, "After receiving the biological material, plant transformation began at the Mahyco Research Facility. After the transformed plants were generated, high performing events were selected and these went into breeding program for transferring the trait to number of genotypes. At this stage, we also started our partnership with ABSP II, which included three public sector partners in India, two in Bangladesh and one in the Philippines. The technology was transferred to their chosen varieties."

"All the regulatory guidelines were followed and all biosafety studies were conducted as stipulated. These studies all require the protocol for conduct of studies be approved by RCGM/GEAC before undertaking the study. All toxicity studies were conducted at third party, government approved facilities. Our team was engaged in the entire process of development of

the product, adds Dr Zehr.

More on Dr Usha Zehr Barwale

- Dr Zehr received her MS (1985) and PhD (1987) in agronomy from the University of Illinois, Urbana-Champaign while working as a research assistant. She earned her BSc (Microbiology) in 1981 from Wilson College, University of Bombay and a diploma in clinical analysis from Sophia College, Bombay in 1982.
- Dr Zehr worked as a geneticist in the Sorghum and Millet Improvement Program at Purdue University, US from 1991-96.
- She serves on a number of boards such as the MS Swaminathan Research Foundation, Barwale Foundation, Sankara Netralaya and Donald Danforth Plant Science Center. She served as a member of the Technical Advisory Committee of the CGIAR in 1997-2002. She is a member of other committees such as the Private Sector Committee of the CGIAR, Intellectual Property Committee of the International Seed Federation, National Biotechnology Network, World Water Commission and Department of Biotechnology, Government of India. She is also director of the Mahyco Vegetables Seeds, Jalna.
- She has been involved in the founding or functioning of several charitable organizations such as Shri Ganapati Netralaya at Jalna, Maharashtra.



Dr Ponnuswami Balasubramanian, *principal investigator and former director, Center for Plant
Vadu Agricultural University (TNAU), Coimbatore*

An eminent agri-scientist and expert, he was instrumental in evolving Bt brinjal with funds from DBT

The role of Dr P Balasubramanian involved the interfacing between his group at TNAU on one side and DBT and ABSPII (USAID) on the other. As per the MOU signed between Mahyco and TNAU, seeds of the target varieties were sent to Mahyco for crossing TNAU varieties with the Mahyco line harboring the EE1 event. Then the F1 seeds of the respective varieties were received by TNAU at TNAU greenhouses using TNAU target varieties as recurrent parents.

The idea was to select the best from among the backcross populations expressing the insecticidal protein at the required level while the original genetic backgrounds of the target TNAU varieties were retained as well. Theoretically, a population of BC3F2 (third back cross population selfed twice) would do for this purpose, says Dr Balasubramanian

While explaining his role, he says he had to submit the reports on ongoing work to appropriate reviewing authorities at the university (Institutional Biosafety Committee) level and national (Review Committee on Genetic manipulation, Genetic Engineering Approval Committee, DBT Task Force Meetings) levels and international (ABSPII) levels. Awareness campaigns for public awareness had to be conducted with generous support from Biotech Consortium of India Limited and ABLE.

More on Dr Ponnuswami Balasubramanian

- Dr Balasubramanian is the former director of Center for Plant Molecular Biology (CPMB) and was involved in establishment of Rice Transformation Facility at TNAU in 1996 with financial support from the Rockefeller Foundation New York.
- DBT with its funding assigned him the work on cloning antifungal genes from *Trichoderma* spp. for use against *Rhizoctonia solani*, the invincible soil-borne plant pathogen.
- He has worked in evolving several transgenic lines of elite rice cultivars of Tamil Nadu expressing resistance to sheath blight and bacterial blight by pyramiding genes encoding a chitinase, a thaumatin-like protein and Xa21. He has built two Transgenic Greenhouse Facilities at TNAU with funding from the Government of Tamil Nadu and DBT.
- His transgenic lines are awaiting clearance from DBT for field trials. He specialized in transgenic plant technology and molecular plant pathology and trained at University of California, Davis, John Innes Center, UK and the Scripps Research Institute, La Jolla, USA. He is currently engaged in evolving transgenic lines of papaya expressing resistance against papaya ringspot virus, evolving transgenic banana expressing enhanced shelf life using elite banana cultivars of Tamil Nadu.
- He is the editor-in-chief of a plant molecular biology journal published from Tamil Nadu. He is a member of the Institutional Biosafety Committees of the following research organizations such as, Sugarcane Breeding Institute, Coimbatore; Bharathidasan University, Thiruchirapalli; PSG Institute of Technology; Institute of Forest Genetics and Tree Breeding; Quinquennial Review Team on Spices Research; and Tamil Nadu Biotechnology Board, Coimbatore.

Maharashtra Hybrid Seeds Company (Mahyco), the Indian partner to American seeds giant Monsanto used a DNA construct containing the cry1Ac gene, a CaMV 35S promoter and the selectable marker genes nptII and aad to transform young cotyledons of brinjal plants. A single copy elite event, named EE-1 was selected and introduced into hybrid brinjal in Mahyco breeding program.

Mahyco donated this technology to three institutes-Tamil Nadu Agriculture University (TNAU), Coimbatore; University of Agricultural Sciences (UAS), Dharwad; and Indian Institute of Vegetable Research (IIVR), Varanasi; and has made this technology available to small, resource-poor farmers for free. This was done to ensure the availability of technology and extend its benefits to the largest number of farmers. The major difference between the Mahyco's hybrids and that of the public institutions is that the farmers retain their rights to save their Bt brinjal OPV seeds for the next season.

Bt brinjal was tested for four years at over 60 locations between 2004-08, with multi-location trials conducted by Mahyco, and independently by the Indian Council for Agricultural Research (ICAR) under the All-India Coordinated Research Program "Vegetable Crops (AICRP-VC). Large scale trials were carried out in 2007 and 2008 under the supervision of the Indian Institute of Vegetable Research (IIVR). The results of the agronomic trials have indicated that in Bt brinjal crops show increased marketable yields by 71 percent over non-Bt counterparts. A 60-70 percent reduction in pesticide spraying for FSB control was recorded across the trials. This is reflected in the Economic Threshold Level (ETL) data for FSB, where ETL was crossed on an average of 0.94 times in the case of Bt brinjal plots, whereas this number ranged from 7 to 7.4 for conventional brinjal hybrids and varieties. Putting these benefits together resulted in a net gain of Rs 50,000-60,000 per hectare when Bt brinjal plots were compared to conventional ones.

Bt Brinjal - The journey so far...

2000: Development of Bt brinjal at Mahyco. Brinjal transformation initiated at Mahyco. **Gene:** cry1Ac (B.t.k. strain HD73), nptII, CaMV35S promoter, construct from Monsanto and transformed by Agrobacterium tumefaciens-mediated method.

2000-01: Lab and greenhouse evaluation for efficacy.

2002: Biosafety tests like pollen flow studies, were taken up along with back-crossing program into different hybrid parents initiated.

2004: Multi-locational field trials were conducted in 11 locations with five hybrids [Mahyco's MHB-4 Bt brinjal, MHB-9 Bt brinjal, MHB-10 Bt brinjal, MHB-80 Bt brinjal and MHB-99 Bt brinjal]. This was also the year when Indian Council for Agricultural Research (ICAR) took up trials with the same hybrids under the All India Coordinated Research Project on Vegetable Cultivation in 11 locations.

2005: Three more new hybrids were assessed by the company [MHB-11 Bt brinjal, MHB-39 Bt brinjal and MHB-112 Bt brinjal] and ICAR in the same year in 11 centers.

2006-07: After the permission of the GEAC, the extensive field trials were carried out in Karnataka, Goa, and Maharashtra at five places. Six popular varieties - Kudchi, Malapur, Udupi, Gulla, Rabakali, and Goa 112 - cultivated locally were modified genetically and used in the field trials.

2008: Pollen flow tests and extensive trials.

2009: Detailed compositional analysis. On Oct 14, Bt brinjal was cleared by GEAC for release.

2010: On Feb 10, it was put under moratorium by the government.

Bt brinjal Event EE-1 contains three genes at a single integration site namely:

1. The cry1Ac gene derived from *Bacillus thuringiensis* (Bt) which produces an insecticidal protein. The cry1Ac gene is driven by a viral promoter, the cauliflower mosaic virus (CaMV) 35S promoter. The Cry (crystalline) 1Ac protein (also called Bt protein) encoded by the cry1Ac gene belongs to a diverse family of insecticidal proteins with specific toxicities to certain groups of insects. This Bt protein encoded by the cry1Ac gene is highly specific to lepidopteran insects including fruit and shoot borer (FSB), which is a major pest of cultivated brinjal.
2. The nptII gene which encodes an antibiotic resistance gene (also referred to as a marker), neomycin phosphotransferase, which allowed modified brinjal plant cells to grow in the presence of the kanamycin, and therefore be selected in the genetic engineering process, while inhibiting the growth of non-modified cells. It has no pesticidal properties.
3. The aad gene which encodes another antibiotic resistance gene O-aminoglycoside adenylyl transferase (aad). The aad gene is also a selectable marker but it is not expressed in Bt brinjal plants because it is under the control of a bacterial regulatory sequence that is not active in plants.

Rahul Koul in Gurgaon