

Bt brinjal on the horizon

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In a historic move, India's biotech regulators approved the country's first biotech food crop, a variety of Bt aubergine or eggplant. It was developed by Monsanto's Indian joint venture partner, Mahyco

The India biotech crop economy is expected to be further boosted as the national biotech regulatory agency, the Genetic Engineering Approval Committee (GEAC), gave its approval to the commercial cultivation of the country's first genetically-modified (GM) food crop, brinjal (aubergine) variety, amidst widespread protests by anti-GM activists. This will be the country's and world's first approval of Bt brinjal variety.

The commercial cultivation, however, is on hold for some more time. Though the GEAC has approved the commercial use of the Bt brinjal, things are still hazy on its likely date with the customer's plates. For the environment minister, Jairam Ramesh, has embarked on another long public consultative process before giving the final go ahead for commercial cultivation.

Brinjal is crucial to India's Rs 72,000 crore vegetable economy. Currently, India is the second largest grower of brinjal in the world after China with 26 percent market share. China is ahead by four percent. More than 40 percent of the India's brinjal crop is lost to pest attacks. The Bt brinjal could minimize this loss to single digits and make the country the world's largest grower of brinjal, the "poor man's vegetable." Similar controversies were raised against the introduction of Bt cotton and Indian farmers lost four or five crucial years by the delay. Will Bt brinjal go the same way and Indian farmers end up paying the price in terms of lost incomes?

The Bt brinjal technology, developed by Monsanto, was taken up by Mahyco and two public institutions— The Tamil Nadu Agricultural University (TNAU), Coimbatore and University of Agricultural Sciences (UAS), Dharwad, Karnataka. In addition,

scientists at Cornell University, US, and scientists in the University of Philippines and Bangladesh Agricultural Research Institute (BARI) were also involved in the development of the technology.

Technology march

Bt brinjal was developed by transforming the brinjal proprietary line of Mahyco. According to MK Sharma, managing director, Mahyco Monsanto, the Bt brinjal contains three genes inserted via genetic engineering techniques.

First, the Cry1AC gene, which encodes for an insecticidal protein Cry1AC, was derived from the common soil bacterium *Bacillus thuringiensis* subsp. *kurstaki* (B.t.k). The Cry1AC gene is driven by enhanced CaMV 35S promoter.

Second, the NPTII gene, which encodes the selectable marker enzyme neomycin phosphotransferase II (NPTII), was used to identify transformed cells that contained the Cry1AC protein. It has no pesticidal properties. The NPTII gene is derived from the prokaryotic transposon Tn5.

The third one is the AAD gene, which encodes for the bacterial selectable marker enzyme 3''(9)-O-aminoglycoside adenyl transferase (AAD), allowed for the selection of bacteria containing the pMON 10518 plasmid on media containing spectinomycin or streptomycin. The AAD gene is under the control of a bacterial promoter and hence not expressed in Bt brinjal. The AAD gene was isolated from transposon Tn7.

The Bt Brinjal was produced using *Agrobacterium*-mediated transformation system. The *Agrobacterium tumefaciens* strain LBA4404 carrying the vector pMON 10518 (which carries Cry1AC, NPTII and AAD genes) was used in the transformation process. The T-DNA, which includes Cry1AC, NPTII, and AAD genes, was transferred into the genome of the brinjal cells. The seeds of a proprietary line of Mahyco were used as source material for brinjal transformations.

After the genes were introduced by *Agrobacterium*-mediated transformation, transgenic plants were regenerated by tissue culture, using kanamycin as the selection agent. The development of an improved method for *Agrobacterium*-mediated brinjal transformation was done at Mahyco. The plants, regenerated through tissue culture procedures on media containing kanamycin, were analyzed using ELISA for the presence of Cry1AC protein. The plants expressing Cry1AC proteins were carried forward and analyzed in subsequent generations to identify lines, in which the transgene segregated in the expected Mendelian fashion. Selected lines were also analyzed by southern blot. A single line (event EE-1) was introduced into the breeding program. A PCR based event ID was developed by Mahyco for this unique event EE-1.

The Cry1AC gene in the transgenic Bt brinjal behaved as a single dominant Mendelian factor indicating stable inheritance of the gene in the plant genome. To be active against lepidopteran insects (brinjal fruit and shoot borer; fruit borer) the protein must be ingested. In the insect gut, the protein binds to specific receptors on the insect midgut, inserts into the membrane and forms ion-specific-pores. These events disrupt the digestive processes and cause death of the insect.

"The Cry1AC protein produced in Bt brinjal is non-toxic to non-lepidopteran insects, birds, fish and mammals as these species lack receptors for the proteins on the surface of their gut cells. The acidic medium in gut of these organisms also makes Cry1AC protein inactive. NPTII and AAD proteins are used as a selectable marker and have no pesticidal activity and are not known to be toxic to any species," said C Kameswara Rao, founder of the Foundation for Biotechnology Awareness and Education.

Trials

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.

The stormy atmosphere created by anti-GM activists did not deter the GEAC decision with only three of the 28 members opposing the commercial release of Bt brinjal variety.

Brinjal is one of the few food crops which is believed to be native to the Indian subcontinent. This crop, *Solanum melongena* L, known as brinjal or baingan in India and eggplant/aubergine elsewhere is important to the country's economy. This highly nutritious, low fat, egg shaped product is consumed widely in the country.

Also, brinjal is the second largest vegetable crop grown in India after potato accounting for 11 percent of the annual vegetable production of 81 million tonnes. Just four percent of India's cultivable land area of 166 million hectares is used for vegetable production. India ranks first in the world in the production of okra (lady's finger) and second in cabbage, cauliflower, eggplant, peas, onion and tomato. In potatoes, India ranks third globally in annual production. However, except in the case of tomato, India's average yield per hectare for the most commonly cultivated vegetables is 20 to 30 percent lower than the world average figures. So technological inputs are crucial to reduce losses due to pest attacks and improve yields significantly.

Mahyco's R&D under the guidance of the Department of Biotechnology and the Ministry of Environment and Forests (MoEF), conducted multi-location field trials regularly from 2004 to 2006 to evaluate the efficacy of Bt brinjal in controlling brinjal fruit and shoot borer. Also, the Indian Council of Agriculture Research, New Delhi, independently conducted field trials of Bt brinjal, using its own protocol, under the aegis of AICRP (VC) during Kharif seasons of 2004-05 and 2005-06. The approval of protocols and supervision of trials conducted by Mahyco were assigned to a Monitoring and Evaluation Committee (MEC). Various teams of experts nominated by MEC regularly visited these trials and submitted reports about these trials to RCGM/GEAC through MEC. These trials were generally aimed to assess insect reactions, yield parameters, insecticide usage, and the economics of Bt brinjal.

Fruit harvested from Bt brinjal plants of the Mahyco hybrids were evaluated for their physical characters including shape, size

and color. These observations were made at green house level, in field at 11 different locations during Kharif 2004 and six different locations during Kharif 2005 season. No differences were observed between Bt and non-Bt brinjal fruit with respect to shape, size and color. Further, the Bt entries suffered significantly less damage due to pest when compared with the check and yielded higher number of marketable fruits.

Brinjal economy

India's brinjal economy is estimated to be close to \$2 billion (Rs 9,600 crore) with 1.4 million farmers cultivating it annually in nearly 550,000 hectares (1.4 million acres). Area under brinjal cultivation has grown by 15 percent in the last 10 years but the production has barely increased by 9 percent. A major factor is the repeated attacks by the insect *Leucinoda orbonalis* or fruit and shoot borer (FSB) which increases the input costs and keeps the production down.

So far India has approved only a single GM crop, Bt cotton, which was allowed to be cultivated commercially in 2002. The runaway success of Bt cotton, more than 85 percent shifting to this GM variety, within years of its introduction has definitely influenced the government decision. India's Bt cotton production has more than doubled to more than 3.1 million bales (of 170 kg each) in the last five years. The annual value of the biotech cotton crop, based on the sale price of Bt cotton seeds in 2008-09 was estimated to be Rs 1,500 crore by BioSpectrum.

India's farmers are quick to adapt modern technologies and the success story of Bt cotton is also likely to be repeated in the case of Bt brinjal too and Bt brinjal seed companies could expect annual seed sales in excess of Rs 500 crore in the next few years.

More importantly, now almost 40 percent of the brinjal production in the country is destroyed by the FSB pest. Adoption of Bt brinjal could reduce the waste considerably and brinjal farmers could expect to rake in additional Rs 3,600 to Rs 4,000 crore annually from product sales.

The approval to Bt brinjal was given at a meeting of the GEAC in the Ministry of Environment and Forests in New Delhi on October 14. The decision has to be ratified by the MoEF and notified in the government gazette and licensing arrangements worked out with the technology developers.

The regulatory approval will mark a new beginning for India's \$300 million, biotech seed industry. Nearly a dozen Indian companies and agricultural research institutions have been developing 12 to 15 other GM crops. The regulatory barrier crossed by the country's first biotech food crops will spur more action on this front.

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Narayanan Suresh

Transgenic Bt benefits

The main benefit of Bt technology is the control of the most damaging pest of a particular crop, says C Kameswara Rao

Technologies come with some concomitant and some consequential benefits, both of which should be taken together in assessing the total benefits that accrue. No technology is risk free. Benefits of a technology should hence be weighed against minimal and acceptable risks and a favorable cost-benefit ratio.

Risk assessment, mitigation, and management are at the heart of regulatory processes. Planting a non-Bt refugium along with Bt crops is a means of mitigating the risk of acquired resistance, and so is gene stacking.

The most direct and the most important benefit of Bt technology is the control of the most damaging pest of a particular crop, such as the American bollworm of cotton, stem borers of rice and corn, rootworm of corn, Colorado beetle of potato or stem and fruit borers of aubergine (brinjal). As systemic pesticides, Bt proteins take care of these pests (see Bt brinjal on the horizon, p. 14). The other pests, on which Bt proteins have little or no effect, need to be controlled by pesticide application, preferably as a part of Integrated Pest Management (IPM) practices.

Bt technology imparts only tolerance of the targeted pest of a particular crop and not total resistance to it. In view of the variation in the expression of Bt genes, due to various internal and external factors, two or three pesticide applications are

needed instead of the usual 10 to 20.

Any crop should be grown under optimal conditions to obtain the best benefits from the new technology. In India, cotton is often grown under near impossible conditions, as farmers are lured into growing a cash crop, irrespective of the inappropriate infrastructure, and suffer disastrous consequences. In many developing countries, the record of both the advice given to the farmers and of farmers taking it seriously, is dismal. Some of the benefits of Bt technology are that it results in a healthy crop, with more biomass and more yield. It reduces risk to farm labor involved in pesticide application. It also results in lower concentrations of pesticide residues on the produce and in the environment. Most importantly, the Bt farmer experiences a sense of certainty of the yield with Bt technology than the earlier scenario of 'spray and pray'.

The Bt technology has no gene based influence on crop yield. And contrary to popular belief, the Bt technology does not cause or control any viral. The scenario for farmers can and will improve only when there are better yields which is possible with Bt technology, contrary to what anti-GM activists would ever want to let happen.