DEVELOPMENT OF FRUIT AND SHOOT BORER TOLERANT BRINJAL

INTRODUCTION

Brinjal has been cultivated in the country for the last 4,000 years, although it is often thought of as a Mediterranean or mid-Eastern vegetable. Among the Solanaceous vegetables, brinjal, *Solanum melongena* Linn. is the most common, popular and principal vegetable crop grown in many geographical parts in India. The area under brinjal cultivation is estimated at 0.51 million ha. with total production of 8,200,000 Mt (FAO data, 2005, http://faostat.fao.org/). Brinjal is mainly cultivated on small family farms and it is a source of cash income for resource-poor farmers. This staple vegetable crop is extensively damaged by the insect brinjal fruit and shoot borer (*Leucinodes orbonalis*) and losses range from 50-70%. The young larvae of the pest bore in to petioles and midribs of large leaves and tender shoots causing shoot tips to wilt and later they bore in to flower buds and fruits. The affected fruits loose their market value besides considerable reduction in yield. The pest poses a serious problem because of its high reproductive potential, rapid turnover of generations and intensive cultivation of brinjal both in wet and dry seasons of the year. Farmers use large quantities of chemical insecticides singly or in combination to get blemish free fruits, which fetch premium prices in the market. Around 25 to 80 sprays are undertaken for effective control of brinjal fruit and shoot borer. This practice of indiscriminate use of insecticides leads to build up of pesticide residues in the produce, destruction of beneficial ionsects, pest resurgence, pesticide exposure to farm workers and environmental pollution. To reduce pest-linked damage in brinjal crop as well as to protect the environment from adverse effects of pesticides, deploying the lepidopteran specific *cry1Ac* gene under the control of enhanced CaMV 35S promoter for high level expression in brinjal would provide an effective built-in control for brinjal fruit and shoot borer as a insect resistance management strategy. This would result in bringing down the cultivation costs of brinjal, as contribution of chemical pesticides to brinjal cultivation is sizable.

Bt brinjal being a transgenic food crop, requires environmental clearance under Rules 8, 9, 10 & 11 of the Rules and Procedures notified by the Ministry of Environment and Forests vide Notification no. 1037 (E) dated 05.12.1989. Prior to the deregulation of transgenic fruit and shoot borer tolerant brinjal data and information is necessary to be produced to demonstrate that this Bt brinjal is equivalent to currently grown non-Bt brinjal varieties in composition and agronomic performance and that the Bt protein expressed by the inserted gene causes no adverse effect when consumed by domestic or wild animals and beneficial insects. The bio-safety and environmental issues related to the Bt brinjal were assessed, which includes molecular characterization of induced gene, biochemical characterization of the expressed protein, estimation of the level of the expressed insect control proteins in brinjal and brinjal products, safety of the expressed proteins to non-target organisms, environmental fate of the Bt protein, and agronomic, compositional and food and feed safety evaluation of Bt brinjal compared to non-Bt brinjal.

CHRONOLOGY OF Bt BRINJAL DEVELOPMENT UNDER REGULATORY SYSTEM

2000	-	Brinjal transformation started.
2000-01	-	Greenhouse evaluation.
2002	-	Pollen flow studies- 2 Locations.
	-	Backcrossing program initiated.
2003	-	Acute oral toxicity studies in rats (Intox, Pune).
2004	-	Mucous membrane irritation test in female rabbit (Intox, Pune).
	-	Primary skin irritation test in rabbit (Intox, Pune).
	-	RCGM multilocation field trials-11 Locations, five hybrids (MHB-4, 9, 10, 80 and 99).
	-	Effects on non-target and beneficial insects.
	-	ICAR first year trials with five hybrids (MHB-4, 9, 10, 80 and 99) under AICRP (VC).
2005	-	Sub chronic oral toxicity study in Sprague Dawley rats (Intox, Pune).
	-	Assessment of allergenicity of protein extract using Brown Norway Rats (Rallis, Bangalore).
	-	Responses, as a dietary feed ingredient to common carp (*Cyprinus carpio)* growth performances (Central Institute of Fisheries Education, Mumbai).
	-	IRM workshop and recommendations.
	-	RCGM trials for three new hybrids (MHB-11, 39, 112).
	-	ICAR second year trials for five hybrids (MHB-4, 9, 10, 80 and 99).
	-	ICAR first year trials for three new hybrids (MHB-11, 39, 112).
2006	-	Chemical fingerprinting of Bt and non-Bt brinjal (including alkaloids) (Indian Institute of Chemical Technology, Hyderabad).
	-	Subchronic (90 days) feeding studies using New Zealand rabbit (Advinus Theraputic, Bangalore).
	-	Effect on performance and health of broiler chickens (Central Avian Research Institute, Izatnagar).
	-	Subchronic (90 days) feeding studies in Goats (Advinus Theraputic, Bangalore).
	-	Feeding studies in lactating crossbred dairy cows (G. B. Pant University of Agriculture and Technology, Pantnagar).
	-	Socioeconomic and risk assessment.

Other studies completed

- Germination and weediness studies.
- Aggressiveness studies.
- Soil micro-biota studies (two years).
- Substantial equivalence studies.
- Protein expression studies.
- Baseline susceptibility studies (two years with 29 populations).
- Food cooking and protein estimation in cooked fruits.
- Molecular characterization and even ID.

MOLECULAR CHARACTERIZATION

Bt brinjal was developed by transforming the brinjal proprietary line of Mahyco. Bt brinjal contains the following three genes inserted via genetic engineering techniques:

1. The *cry1Ac* gene, which encodes for an insecticidal protein, Cry1Ac, derived from the common soil bacterium *Bacillus thuringiensis* subsp. *kurstaki* (*B.t.k*). The *cry1Ac* gene is driven by enhanced CaMV 35S promoter.
2. 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 (Beck *et. al*., 1982).
3. The *aad* gene which encodes for the bacterial selectable marker enzyme 3"(9)-O- aminnoglycoside 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* (Fling *et. al*., 1985).

The Bt transgene in the transgenic Bt brinjal behaves as a single gene, dominant Mendelian factor and is stably integrated in the plant genome. To be active against lepidopterans 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. Also 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.

TRANSFORMATION TECHNIQUE USED FOR DEVELOPING Bt BRINJAL

Seeds of a proprietary line of Mahyco were used as source material for brinjal transformations. The *Agrobacterium tumefaciens* strain LBA4404 carrying the vector pMON 10518 (which carries *cry1Ac, nptII* and *aad* genes) was used in the transformation process. The *cry1Ac* gene is under the transcriptional control of the enhanced CaMV35S promoter (P-E35S). The aforesaid genes have been introduced by *Agrobacterium*- mediated transformation, into young cotyledons of brinjal and transgenic plants have been regenerated by tissue culture, using kanamycin as the selection agent. The development of an improved method for *Agrobacterium*-mediated brinjal transformation has been done at Mahyco. This is based on a method that has been described earlier (Fari *et. al*., 1995). 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 is developed by Mahyco for this unique event EE-1.

BIOLOGY OF THE PLANT SYSTEM

Brinjal belongs to the family Solanaceae and is known under the botanical name *Solanum melongena* L. The family contains 75 genera and over 2000 species. There are 3 main botanical varieties under the species *melongena* (Choudhury.1976). The round or egg-shaped cultivars are grouped under var. *esculentum*. The long, slender types are included under var. *serpentinum* and the dwarf brinjal plants are put under var. *depressum*. The common brinjal, to which the large fruited forms belong, is known under the name *S. melongena* var. *esculentum*. Among the 22 Indian species of genus *Solanum*, there is a group of 5 related ones, all prickly and diploids viz., *S. melongena L., S. coagulans (syn: S. incanum L.), S. xanthocarpum, S. indicum L. and S. maccani*. It appears that *S. melongena* is more closely related to *S. incanum* than to any other species. *S. melongena* is readily crossable with *S. incanum*. Somatic chromosome number is 2n = 24.

Brinjal plant is usually self-pollinated, but the extent of cross-pollination has been reported as high as 48% and hence it is classified as often cross-pollinated crop. Brinjal is often cross-pollinated due to heteromorphic flower structure called as heterostyly. Outcrossing primarily takes place with the help of insects.

EFFICACY OF BT BRINJAL AGAINST TARGET PESTS

Efficacy studies were conducted by Mahyco. Insecticidal activity of the transgenic Bt brinjal against brinjal fruit and shoot borer (*Leucinodes orbonalis*) and *Helicoverpa armigera* was assayed. Bt brinjal was found to be effective against these target pests. Insect mortality of 98% for FSB was observed in the transgenic Bt brinjal shoots, whereas in the control shoots, mortality was < 30%. The fruit bioassays results demonstrate that transgenic brinjal fruits are resistant to Leucinodes, as the mortality rates of the larvae are very high (upto 100%) when compared with non-transgenic control plants. The results of leaf and fruit bioassays against *Helicoverpa armigera* indicates that the Bt brinjal leaves and fruits are highly resistant (99%) to Helicoverpa.

ENVIRONMENT & BIOSAFETY INVESTIGATION CARRIED OUT TO ASSESS Bt BRINJAL

A. Environmental effect

(i) Pollen escape

Pollen flow studies on Bt brinjal were conducted by Mahyco at two different locations (Jalna, Maharashtra and Ranebennur, Karnataka) during Kharif 2002. Central block containing Bt brinjal was surrounded by concentric rings of non-Bt brinjal to assess the distance travelled by the transgene and the outcrossing percentage. Pollen flow studies at two locations show that at Jalna (Maharashtra) maximum distance that the pollen traveled was 20 meters, 10 out of 681 progenies showing the presence of the gene giving a outcrossing percentage of 1.46%. At Ranebennure (Karnataka), maximum distance that the pollen traveled was 15 meters and 18 progenies out of 663 show outcrossing (2.7%).

(ii) Germination, Aggressiveness & Weediness

To assess the weediness of Bt brinjal, the rate of germination and vigor was compared by laboratory test and in soil to the non-transformed counterpart. The results demonstrated that there are no substantial differences between Bt and non-Bt brinjal for germination and vigor. This also indicates that there is no substantial difference between transgenic Bt and control non-Bt brinjal with regard to their weediness potential.

Also a field study was conducted by Mahyco to monitor the aggressiveness of Bt brinjal as compared to its non-Bt counterparts. After complete harvesting of the brinjal crop, the area under planting of Bt brinjal at Jalna, Maharashtra was left undisturbed and irrigated on a regular basis to allow for germination of any seeds that might have remained in the ground after harvesting the main crop (plot was observed up to 3 months after final harvesting). The data provides information on germination rates and aggressiveness under field conditions of naturally shed brinjal seeds in the plots where Bt and non-Bt plants had been grown. If any plant growth occurred, the same was checked with ELISA to determine if it was transgenic or not.

There was no brinjal plant observed to grow or germinate in this plot for the period of the study. The data suggest that there is no aggressiveness or weediness demonstrated by of Bt brinjal plants. Bt brinjal does not have any weediness/aggressiveness characteristics and behaves in a similar fashion as other conventional brinjal varieties. Brinjal is not considered to have weediness characteristics, such as seed dormancy, soil persistence, germination under diverse environmental conditions, rapid vegetative growth, a short life cycle, high seed output and dispersal. Growth and development of Bt brinjal were routinely monitored in all the field and greenhouse trials. Bt brinjal does not exhibit any different agronomic or morphological traits compared to non-Bt brinjal/controls that may give it a competitive advantage over other species in the ecosystem in which it is grown.

SOIL ANALYSIS

It was important to assess the possible risk of accumulation and persistence of the plant produced Bt proteins in soil where the crop are repeatedly grown and plant residues such as roots are ploughed back into soil. The issue of the impact of the Bt protein released in to the soil on soil organism is an important one. To address such issue Mahyco R&D conducted soil studies in years 2003-04 (at Jalna) and 2004-05 (7 locations) by collecting soil samples from selected locations periodically.

The effect of growing Bt brinjal in open field, on soil microflora, residue of Cry 1Ac protein and soil invertebrates was studied. It was clearly demonstrated that there were no differences between Bt and non-Bt plots vis-à-vis soil bacteria and fungal count both at the rhizosphere and the soil beyond the rhizosphere.

(i) Rapid degradation of Cry 1 Ac protein in soil

The level of Bt protein in soil samples was determined by Insect bioassays. Regarding the residual Bt protein in the soil, after harvest of the crop it was found to be non-detectable in any of the soil samples tested. These results are consistent with the

literature report and that the Bt protein is rapidly degraded in the soil and therefore, there is no accumulation of the protein in the soil associated with production of Bt brinjal.

(ii) Soil Micro flora

For analyzing any impact of Bt protein leached by roots of Bt brinjal plant, it was assessed by culturing bacteria and fungi from collected soil samples by dilution planting method. ANOVA analysis of the microbial population showed no significant difference between Bt & non-Bt soil samples. Similarly no significant variation was observed in the population of soil invertebrates like Earthworms & *collembola.*

SUBSTANTIAL EQUIVALENCE STUDIES OF Bt BRINJAL

Substantial equvalence studies were conducted by Mahyco, Kallakal (AP). Protein, carbohydrate, oil, calories, ash, nitrogen, crude fibers and moisture contents were analyzed. A comparative study for the chemical composition of the tissues of brinjal plants was made using transgenic Bt brinjal and three non-Bt controls. The chemical composition was determined in the fruit, leaf, stem and root tissues of the brinjal plant. No statistical differences between Bt brinjal and non-Bt brinjal groups were observed in the chemical constituents of moisture, proteins, oil, ash, carbohydrates, calories for fruit tissue and nitrogen, ash and crude fiber contents in leaf, stem and root tissues.

CRY1AC PROTEIN EXPRESSION AND QUANTIFICATION

These protein studies were conducted by Mahyco Research Center, Dawalwadi (Maharashtra). Quantitation of Cry1Ac insect control protein in various tissues of eight Mahyco brinjal hybrids was done. The concentrations of in-planta expressed Bt insecticidal protein, Cry1Ac in various tissues (leaf, shoot, stem, flower, fruit and root) were quantified using a quantitative enzyme-linked imunosorbent assay (ELISA). Tissues from non-Bt of each hybrid were used as control tissues in the assay. Cry1Ac was not detected in any of the non-Bt samples. The levels of Cry1Ac protein concentrations were consistent with and sufficient for effective control of brinjal fruit and shoot borer (BFSB), *Leucinoides orbonalis*. The levels of Cry1Ac protein was found to vary between 5 to 47 ppm in shoots and fruits.The values of Cry1Ac content in various tissues and their efficacy in BFSB control can be placed in the context of the mean molt inhibitory concentration (MIC_{95}). MIC_{95} for *Leucinoides orbonalis* was calculated to be 0.059 ppm for Cry1Ac. Clearly, all the hybrids over all locations and the entire life of the crop expressed Cry1Ac insecticidal protein well above the MIC_{95} value.

BASELINE SUSCEPTIBILITY STUDY

Mahyco R&D carried out this studies consecutively for two years in 2004-05 & 2005-06. Brinjal fruit and shoot borer, *Leucinodes orbonalis* Guen. (Lepidoptera: Pyralidae), infested fruits were collected from fields. There were a total of twenty nine locations which included nine populations collected from RCGM Bt brinjal trial locations in Kharif 2004, six populations from RCGM Bt brinjal trials in Kharif 2005 and fourteen populations during 2004-'05.

The Cry1Ac susceptibility data for *L. orbonalis* populations collected from different locations showed 12-fold variability in LC_{50} value of all twenty nine populations tested for Cry1Ac susceptibility. The highest LC_{50} was observed at Ahmednagar, Maharashtra (0.095 ppm of diet). The LC_{95} values followed similar trend of 13.5-fold variability. The field populations demonstrated 70-fold inter population variation in the insect susceptibility to the Cry1Ac protein indicated by MIC_{50}. The variability was 14-fold when MIC_{95} was considered and values ranged from 0.020-0.138 ppm of diet. Average MIC_{95} was found to be 0.059ppm. There was 100% mortality among most populations at the highest concentration used in the bioassays.

FOOD COOKING AND PROTEIN ESTIMATION IN COOKED FRUITS

Food cooking studies and protein estimation in cooked fruits were done at Mahyco Research Center, Dawalwadi (Maharashtra). Cooked brinjal fruits are consumed in various forms in India. Tender Bt brinjal fruits were used in these studies to determine whether the Bt protein was present in the cooked fruits. The Bt protein was undetectable in the cooked fruits at the first sampling time-point irrespective of the cooking method used (roasted, shallow-fried, deep-fried or steamed). The first sampling time-point was 5 min for roasted fruit and 1 min for the other forms of cooking. This study indicates that the Cry1Ac protein in Bt brinjal fruits is rapidly degraded upon cooking.

RELATIVE TOXICOLOGICAL & ALLERGENICITY ASSESSMENT OF Bt BRINJAL USING ANIMAL MODELS

(i) Toxicological Studies

- Acute oral toxicity study of transgenic Bt brinjal was conducted at INTOX PVT. LTD., Pune, Maharashtra, India to assess the safety of Bt brinjal. Acute oral administration of transgenic Bt brinjal expressing Cry1Ac protein to Sprague Dawley rats at the limit dose of 5000mg/ kg did not cause any toxicity. Proteins that are non-toxic by the oral route are not expected to be toxic by the dermal or pulmonary route.

- Subchronic oral (90 Days) toxicity study of transgenic Bt brinjal in Sprague Dawley Rat was conducted at INTOX PVT. LTD., Pune, Maharashtra, India. Based on the findings of this study, the no-observed-adverse-effect-level (NOAEL) of transgenic Bt brinjal expressing Cry1Ac protein in Sprague Dawley rat, following oral administration for 90 days was found to be more than 1000 mg/kg body weight. This study demonstrates that Bt brinjal expressing Cry1Ac protein is non-toxic to the study animal by oral route.

(ii) Allergenicity Studies

Assessment of the allergenicity of protein extract from transgenic Bt brinjal was conducted at Rallis India Limited, Bangalore, India. The objective of this study was to assess the relative allergenicity of transgenic Bt brinjal compared to the allergenicity of conventional brinjal (non-transgenic), as measured by active cutaneous anaphylaxis (ACA) in Brown Norway Rats sensitized with brinjal. Six to seven weeks old Brown

Norway Rats were randomly selected and used for the studies. The animals were observed daily for signs of toxicity and pre-terminal deaths, weekly body weights and food consumption.

There were no clinical signs of toxicity and pre-terminal deaths (mortalities). The weekly mean body weights were increased in all the groups. There was no statistically significant intergroup difference in body weights between treatment and control groups. There were no significant differences in food consumption between treatment and control groups.
There were no differences between the skin reactions of each of the 4 extracts on the same animals. These observations suggest that there are no differences between the allergenicity or inflammatory characteristics of the 5 brinjal extracts tested including transgenic Bt brinjal and non transgenic brinjal.

Statistical analysis of this study concluded that there are no biological differences between the allergenicity response amongst all the brinjal hybrids including transgenic Bt brinjal and non-transgenic brinjal.

(iii) Primary skin irritation test in rabbit

Primary skin irritation test of transgenic Bt brinjal in rabbit was also conducted at INTOX PVT. LTD., Pune, Maharashtra, India. Transgenic Bt brinjal expressing Cry1Ac protein applied to intact rabbit skin for 4 hours did not cause any skin reaction throughout the observation period. The irritancy index was also 0.0. The observations and results of this study leads to the conclusion that transgenic Bt brinjal expressing Cry1Ac protein can be classified as non-irritant to skin in rabbit.

(iv) Mucous membrane irritation test in female rabbit

Mucous membrane irritation test of transgenic Bt brinjal in female rabbit was conducted at INTOX PVT. LTD., Pune, Maharashtra, India. Application of transgenic Bt brinjal expressing Cry1Ac protein to the vaginal mucous membrane of the female rabbit did not cause any erythema or edema as observed for 72 hours after application. Based on the average irritation index (0.0), transgenic Bt brinjal expressing Cry1Ac protein was classified as non-irritant to mucous membrane in rabbit.

ALKALOID CONTENT COMPARISON IN BT AND NON-BT BRINJAL

Isolation and identification of major alkaloid principles in Bt and Non-Bt counterpart hybrids was carried out in fruits and roots by the Indian Institute of Chemical Technology, Hydrabad. The residue extraction and separation was carried out by using approved protocol and the same was chromatographed over silica gel and eluted to obtain two alkaloids namely Solamargine (mol wt 867) and Solasonine mol wt 883). The structure of alkaloids was identified based on extensive 1-D and 2-D NMR and other spectroscopic studies. It appears from the present study that the alkaloid profile from powder samples of fruit and roots of Bt and non-Bt Solanum melongena are the same with not much of appreciable variation in their relative abundances.

NUTRITIONAL STUDIES

Food products derived from brinjal are extensively processed before the use for human consumption. Therefore, no intact protein or genetic materials are expected to be contained in food products derived from brinjal. Brinjal has a history of safe use as a source of food in India. Brinjal is a highly productive crop, the fruit are consumed as cooked vegetables in various ways, and dried shoots are used as fuel in rural areas. Brinjal is a good source of minerals and vitamins, and rich in total water soluble sugars, free reducing sugars, amide proteins among other nutrients. The fundamental principal of substantial equivalence when applied to Bt brinjal and its non-Bt counterpart has revealed that Bt brinjal is substantially equivalent in its composition to control brinjal and thus the food and feed derived from Bt brinjal will also be substantially equivalent to food and feed derived from non-Bt counterpart. In addition to compositional analysis the wholesomeness of feed from Bt brinjal was demonstrated in separate feeding studies with fish, chickens, cows, goats and rabbits.

(i) Rabbits

Subchronic (90 days) rabbit feeding studies were conducted on New Zealand White rabbits at Advinus Therapeutics Private Ltd., Bangalore, India. The objective of this study was to compare the wholesomeness and safety of transgenic Bt brinjal containing *cry1Ac* gene with control non-Bt brinjal. As per the findings of this study, it was concluded based on the health, growth and physio-pathological parameters analyzed during the experiment that there were no significant differences between the groups fed with transgenic Bt brinjal containing *cry1Ac* gene and control non-Bt brinjal fruit.

(ii) Fish

Fish (common carp, *Cyprinus carpio*) feeding study was conducted at Central Institute of Fisheries Education, Mumbai, India. The objective of this study was to evaluate the effect of genetically modified Bt brinjal expressing *cry1Ac* gene, as a feed ingredient for common carp and to study the comparative growth and survival of fish on feeding Bt brinjal as compared to non-Bt counterpart and non-Bt commercial checks.

The result showed that there were no significant differences in terms of growth patterns, food conversion ratio, feed efficiency ratio and protein efficiency ratio among different Bt and non-Bt brinjal treatments fed to fish for 45 days. Different Bt and non-Bt brinjal treatments were statistically similar on the basis of isocaloric and isoproteinaceous feeds in terms of fish growth responses, and histopathological alterations in gill, liver, intestine and kidney tissues in common carp.

(iii) Chicken

A chicken feeding study was conducted at Central Avian Research Institute, Izatnagar, India. The objective of this study was to assess the impact of transgenic Bt.brinjal expressing *cry1Ac* gene on chickens, in terms of growth performance and nutrient utilization. Results of the present study showed that body weight gain, feed intake and feed conversion ratio did not differ among Bt and non-Bt treatments after addition of dried Bt and non-Bt brinjal in the trials at 5 or 10% levels of iso-caloric diets. Several blood biochemical constituents did not differ statistically due to dietary treatments including Bt and non-Bt brinjal incorporated diets. This study found Bt brinjal to be as safe as non-

transgenic brinjal in terms of responses of chickens fed with diet incorporating the two types of brinjal. This study also state that brinjal is a moderate energy rich feedstuff and can be safety incorporated upto 10% level in maize soy based broiler rotation.

(iv) Goats

Subchronic (90 days) goat feeding studies were conducted at Advinus Therapeutics Private Ltd., Bangalore, India. The objective of this study was to compare the wholesomeness and safety of transgenic Bt brinjal containing *cry1Ac* gene with control non-Bt brinjal. As per the results of this study, it was concluded based on the health, growth and physio-pathological parameters analysed during the experiment that there were no significant differences between the groups fed with transgenic Bt brinjal containing *cry1Ac* gene and control non-Bt brinjal fruit.

(v) Cows

Cow feeding studies were conducted at G. B. Pant University of Agriculture and Technology, Pantnagar to assess the nutritional value of transgenic Bt brinjal fruit in comparison to non-transgenic (non-Bt) brinjal fruit in lactating crossbred cows in terms of feed intake, milk production and milk composition and to determine if the Bt. Protein was detectable in milk and blood of lactating crossbred cows fed ration containing transgenic Brinjal fruits. From the present studies, it was concluded that the nutritional value of both transgenic and non-transgenic brinjal fruits were similar in terms of feed intake, milk yield and milk constituents without any adverse affect on health of lactating crossbred cows.

RESISTANCE MANAGEMENT STRATEGIES FOR Bt BRINJAL

To achieve the agronomic benefits provided by Bt brinjal, it is important that brinjal with Bt gene be deployed and managed to sustain the technology. This can only be achieved by implementation of integrated pest management technique and use of strategies to delay the development of insect resistance to Cry1Ac protein.

To address the possible strategies that could be employed to reduce the likelihood of target insects developing resistance to the Cry1Ac protein in India, Mahyco scientists have collaborated closely with leading pest and resistance management researchers from academia, government and extension. In collaboration with the experts (Cconsultation with TNAU, UAS Dharwad, Mahyco, Cornell Univ, Univ. of Philippines, BARI and East West Seeds), computer simulations and laboratory and field studies have been conducted to evaluate strategies for managing caterpillar resistance to the Cry1Ac protein. Results from these experiments, combined with an understanding of brinjal production and agronomic practices, provide the basis for a sound, practical, resistance management program. As a result of these efforts, the following have been identified as key resistance management strategies for the Bt gene in India:

1) Monitoring for baseline susceptibility.
2) Resistance monitoring.
3) Assessment of level of control.
4) Refuge design and placement.
5) Remedial Action Plan.
6) Encourage integrated pest management (IPM):

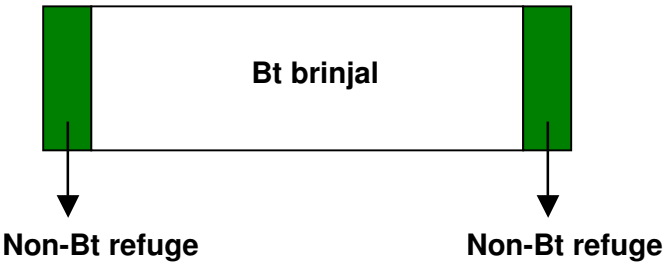
7) Farmer field days and educational programs.

Structured refuge in which 5% of the plants in the field would be non-Bt:
The rationale for 5% is as follows:

There are approximately 20,000 brinjal plants per ha and a 5% refuge would require 1000 plant/ha. A conservative estimate would be that there are 8 fruit per plant and that, if infested, each fruit would have 3 larvae. The potential for the number of adults produced per plant would be 24, or 24,000 per 1000 refuge plants. This figure represents the potential number of adults produced per picking, and there may be an average of 15 pickings per plant (=360,000 adults produced per 1000 refuge plants during a season). The US Environmental Protection Agency suggests a goal of the refuge should be to produce 500 adults from the refuge for every survivor in the Bt crop. If the refuge plants are treated with an insecticide, this will reduce the number of adults produced. However, it was the consensus of the group that such sprays would still allow 50% of the BFSB to survive. Even if there were 48 survivors/ha on the Bt plants per picking (or 720 during the season), this ratio of 500:1 would be upheld.

No survivors have yet been detected in the Bt brinjal, suggesting that this ratio would be upheld. Assuming field tests show no BFSB survival to adulthood, seeds will be packaged so, for example, each 95 grams of Bt seeds will come with another package of 5 grams of non-Bt seed. Because BFSB adults in the refuge should be encouraged to mate with any survivors on the Bt brinjal (i.e. random mating), it is suggested that for every 0.5 acre planting of Bt plants there should be 0.025 acres of non-Bt plants for the refuge. Farmers will be advised to plant on 2 sides of the Bt brinjal block to “bracket” it and thereby encourage moths to move freely across the field and promote random mating within the population.

Diagram showing IRM model for Bt brinjal



(Bt brinjal in the middle section with non-Bt refuge plants on the sides)

EFFECT OF Bt PROTEIN ON NON-TARGET ORGANISMS

Mahyco R&D conducted multi location Field trials during the years 2004-05 & 2005-06. The protocol adopted to conduct these trials had specific mention of the assessment of the effect of Bt brinjal on non-target pests (sucking pest, secondary lepidopterans) and beneficial insects of brinjal crop. The vast data collected in all these years from various locations showed that non-target sucking pest counts (aphids, jassids, white fly, leafhoppers & thrips) did not vary significantly among Bt and non-Bt brinjal hybrids.

The beneficial insects namely Chrysopa, lady beetle and spiders were also observed to be active in both Bt and non-Bt brinjal crops.

MAHYCO BRINJAL HYBRID FRUIT CHARACTERISTICS

There is a distinct regional consumer preference for the various types of brinjal fruit depending upon shape and colour of the fruit, calyx characteristics. MHB-4 is preferred in Gujrat, Maharshtra, Bihar Jharkhand and UP. MHB-9 is preferred in A.P., Karnataka, T.N. and parts of Maharashtra. MHB-10 is preferred in Maharashtra, Karnataka, Rajasthan, M.P., Bihar, Jharkhand, parts of A.P. and Gujarat. MHB-11 is preferred in Gujrat, M.P., Maharashtra, T.N., parts of Karnataka and A.P. MHB-39 has wide preference in Gujrat, Rajasthan, M.P., Maharashtra, T.N., Pondicherry, Bihar, Jharkhand, A.P., Karnataka and Orissa. MHB-80 is Bharta type and preference is widely distributed throughout India. MHBJ-99 is preferred in parts of A.P., Maharashtra, T.N. and Karnataka. MHB-112 is widely preferred in north India.

Fruit harvested from Bt brinjal plants of the Mahyco hybrids were evaluated for their physical characters including shape, size and colour. These observations were made at green house level, in field at 11 different locations during Kharif 2004 and 6 different locations during Kharif 2005 season. No differences were observed between Bt and non-Bt brinjal fruit with respect to shape, size and colour.

Fruits from different Bt hybrids are characterized in the table below-

Hybrids	Fruit colour	Fruit shape	Calyx colour	Spinyness on calyx
MHB-4	Green + White + Purple stripes	Oblong	Green	Spiny
MHB-9	Green	Elongated	Green	Non-spiny
MHB-10	Purple + white	Slight oval	Green	Spiny
MHB-11	Purple + white	Oval	Green	Non-spiny
MHB-39	Shining reddish purple	Oval	Green	Non-spiny
MHB-80	Blackish Purple	Round	Green	Non-Spiny
MHBJ-99	Green + white	Oval	Green	Non-spiny
MHB-112	Black	Oblong	Green	Non-Spiny

AGRONOMIC EVALUATION OF Bt BRINJAL

To evaluate the efficacy of Bt brinjal in controlling brinjal fruit and shoot borer, Mahyco's R&D under the guidance of the Department of Biotechnology, Ministry of Environment and Forests conducted multi-location field trials regularly from 2004 to 2006. Also on the advice of MoE&F, Indian Council of Agriculture Research, New Delhi conducted field trials of Bt brinjal independently, using their own protocol, under the aegis of AICRP (VC) during Kharif seasons of years of 2004-05 and 2005-06.

Years	Mahyco	ICAR
2004-05	Multilocation (11 locations) trials (five hybrids)	1st yr trials (12 centers) (five hybrids)
2005-06	---	2nd yr trials (11 centers) (five hybrids)
2005-06	Multilocation (6 locations) trials (additional 3 hybrids)	1st yr trials (11 centers) (additional 3 hybrids)

RCGM of DBT and GEAC of MoE&F assigned the approval of protocols and supervision of trials conducted by Mahyco, to Monitoring and Evaluation Committee (MEC). Various teams of experts nominated by MEC regularly visited these trials and submitted their reports about these trials to RCGM/GEAC through MEC.

These trials were generally aimed to assess the following parameters –

A. Insect reactions
(i) Shoot Damage
(ii) Fruit borer larvae
(iii) Fruit Damage
(iv) Stem borer damage
(v) Sucking pest infestation
(vi) Beneficial insects

B. Yield Parameters
(i) Number of healthy (marketable) fruits and those damaged by borers.
(ii) Weight of healthy (marketable) fruits and those damaged by borers.

C. Insecticide usage
(i) Sprays for fruit and shoot borer based on ETL in Bt, non-Bt counterpart & check.

D. Economics of Bt brinjal
(i) Savings on number of sprays
(ii) Yield benefit due to protection against fruit and shoot borer

Based on results from Field evaluation of Bt brinjal at 11 locations during K-04 and 6 locations during K-05 show that the Bt entries suffered significantly less damage due to pest when compared with the check and yielded higher number of marketable fruits. For BFSB related observations, significant differences were detected between hybrids based on presence or absence of Bt gene. For BFSB count, significant differences were detected between Bt hybrids (containing *cry1Ac* gene) and all non-Bt checks. All Bt

hybrids were significantly lower in number of BFSB larvae. Differences were also measured between the Bt hybrid and non-Bt check hybrids for shoot damage to plants from BFSB infestation. Percent damage to shoots were significantly lower for the Bt group as compared to non-Bt hybrids. The degree of such differences in BFSB feeding damage between Bt hybrids and non-Bt hybrids was significant. It was presented that the average shoot damage in Bt. brinjal hybrids ranged from 0.04 to 0.3% as compared to 0.12 to 2.5% in non-Bt. brinjal hybrids and the percentage of damaged fruits ranged from 2.5 to 20% in Bt. entries as compared with 24 to 58% in non-Bt. counterparts. Number of larvae in Bt. entries per plant ranged from 0 to 20 as compared with 3.5 to 80 larvae in non-Bt entries and significant yield increase in Bt. brijnal hybrids as compared to the non-Bt counterpart was noted. No significant differences were seen for non-target pests and beneficial insects. It was concluded that the target pest is controlled by Bt. brinjal and biosafety studies conducted till date show no significant differences between Bt and non-Bt brinjal

SOCIO ECONOMIC AND RISK ASSESSMENT STUDIES

Detailed socioeconomic studies will be conducted along with large scale trials of Bt brinjal. However published studies by Chong M., Journal of Risk Research 8(7-8), 2005 and Krishna V. and Qaim M, unpublished (2005) which indicate the potential of Bt brinjal to increase farmer's welfare through insecticide reductions, and an increase in marketable yields of brinjal fruits.

Different studies were conducted separately by University of Hohenheim, Stuttgart, Germany and Singapore Management University, Singapore to demonstrate socio-economic impact of Bt brinjal. These studies were based on interviews of different stakeholders related to brinjal crop, statistical analysis of field evaluation data of Bt brinjal and statistical models to assess adoption potential of Bt technology. These studies demonstrated the potential of Bt brinjal to offer economic benefits over conventional brinjal cultivation. Field trial data show that Bt technology has a significant potential to increase farmers' welfare through insecticide reductions and sizeable increases in marketable yield. The findings indicate that economic benefits, safety concerns, and accountability are most salient to Indian farmers' perception of the risks and benefits of Bt eggplant. Significantly, in one of the studies, none of the farmers mentioned moral concerns as an issue. The findings also make clear that economic benefits outweigh perceived risks. This study concludes that economic benefits are more salient than moral concerns to Indian farmers' perception of Bt eggplant. Also, innovative models of public-private partnership, like the one of Bt brinjal in India, can be beneficial for all parties involved.

CONCLUSIONS

The target pest is controlled by Bt brinjal.

Bio-safety studies conducted have shown no significant differences between Bt and non-Bt brinjal.

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