

Development and Assessment of Fruit and Shoot Borer-Resistant Bt Brinjal

Maharashtra Hybrid Seeds Company Ltd
Mumbai

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Rationale for development: the problem of FSB

- ☐ Brinjal is one among a number of key crops which are extensively prone to infestation and damage by lepidopteran class insect pests.
- ☐ Fruit and shoot borer (FSB) is the main pest problem in brinjal production.
- ☐ Yield losses estimated to be 60 to 70% even after repeated insecticide sprays, with documentation of >70 sprays in a single season (AVRDC).
- ☐ Current control methods involve intensive use of pesticides due to the biology and feeding behaviour of FSB.
- ☐ High use of pesticides on brinjal has resulted in higher cost of production, environmental pollution, destruction of natural enemies and health problems due to pesticide residues.
- ☐ Conventional sources of resistance are not available to breeders and thus there is a need for alternatives.



Overview

- Transformation experiments and greenhouse evaluation initiated in 2000 at Mahyco from which event EE-1 was chosen
- Public private partnership entered into with TNAU, Coimbatore and UAS, Dharwad and IIVR, Varanasi
- Event EE-1 expresses the *cry1Ac* gene under a constitutive promoter to produce a lepidopteran-specific insecticidal protein isolated from *Bacillus thuringiensis*
- Biosafety studies and field trials (~60) undertaken over 2002-2009 as per the protocols prescribed by RCGM
- Based on the biosafety data and results of multi-locational trials, RCGM had recommended Bt brinjal for large-scale trials to GEAC in 2006



Overview

- Expert Committee –I reviewed the biosafety data and recommended additional studies in 2007
- GEAC approved the conduct of large scale trials and biosafety studies under the supervision of Director, IIVR
- Following the completion of LST and biosafety studies an Expert Committee –II was constituted which examined the biosafety data and issues raised by NGOs
- Biosafety data was made available in the public domain



Safety Assessment

Characterisation of the genetic modification

- Source of genes
- Molecular characterisation
- Stability
- History of safe use and consumption
- Function and specificity of the expressed protein
- Expression levels through the crop cycle



Safety Assessment

Food and feed safety

- ☐ Toxicity testing
- ☐ Allergenicity testing
- ☐ Proximate analysis
- ☐ Detailed compositional analysis
- ☐ Animal feeding safety assessment (fish/bird/mammals)
- ☐ Effect of cooking

Environmental safety

- ☐ Potential of gene transfer to related species
- ☐ Pollen flow and outcrossing
- ☐ Safety to non-target organisms
- ☐ Persistence in soil
- ☐ Weediness



Outcome of assessment

- ☐ The inserted genes and regulatory sequences have a history of safe use
- ☐ The expressed Bt protein is highly specific to lepidopteran pests
- ☐ Expression of the *cry1Ac* gene is stable during the life of the crop and the levels of Cry1Ac protein are sufficient for effective control of FSB in the agro-climatic conditions tested
- ☐ The Cry1Ac protein expressed in Bt brinjal is 100% identical to that in Bt cotton
- ☐ Introgression of the *cry1Ac* gene has not altered outcrossing and weediness characteristics



Outcome of assessment

- No adverse impact on non-target organisms including beneficial organisms and soil micro-flora
- No instances of natural inter-specific hybridization with wild species reported for cultivated brinjal
- Cry1Ac protein is neither toxic nor allergenic to human and animals. It rapidly degrades in 30 seconds in simulated gastric and intestinal fluids and also upon cooking
- Studies indicate potential enhanced economic benefits to farmers resulting from higher marketable yield and lower use of pesticide sprays



Chronology

2000	Brinjal transformation started
2000-01	Greenhouse evaluation
2002	Pollen flow studies- 2 Locations Backcrossing program initiated
2003	Acute oral toxicity studies (rats)
2004	Mucous membrane irritation test (rabbit) Primary skin irritation test (rabbit) RCGM-MLT, ICAR trials



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| 2005 | Subchronic oral toxicity study
Allergenicity study (rat)
Fish feeding study (fish)
RCGM-MLT, ICAR trials, IRM workshop |
| 2006 | Chemical fingerprinting
Subchronic feeding studies (goat/rabbit)
Chicken feeding studies
Lactating cow feeding studies
Socioeconomic and risk assessment |
| 2007 | Large Scale Trials for seven hybrids
Pollen flow studies at two locations
Crossability study |
| 2008 | Second year Large Scale Trials for seven hybrids
Pollen flow studies at two locations.
Crossability study |
| 2009 | Detailed compositional analysis |
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Biosafety studies

1. Germination

- ✓ Tests conducted using paper towel/soil
- ✓ No significant difference in germination among Bt and non-Bt counterparts

2. Weediness and aggressiveness

Fields monitored for up to three months after crop harvest indicate that Bt brinjal does not show any aggressiveness or weediness

3. Substantial equivalence

- ✓ Percent Moisture, protein, oil, ash, carbohydrates and calories were estimated in fruit, leaf, stem and root tissues of Bt and non-Bt brinjal
- ✓ No significant differences in above parameters were observed

4. Chemical fingerprinting studies (IICT, Hyderabad)

- ✓ Alkaloids Solamargine and Solasonine were estimated
- ✓ For alkaloids detection, TLC and HPLC profiles of Bt and non-Bt brinjal are similar
- ✓ No appreciable variation in relative abundance of alkaloids between Bt and non-Bt tissues

5. Cooking studies No Cry1Ac protein was detected in cooked Bt brinjal fruits



6. Acute oral toxicity study (Intox, Pune)

In male and female rats - Acute oral administration of Bt brinjal to Sprague Dawley rats did not cause any toxicity.

7. Sub chronic oral (90 day) toxicity study (Intox, Pune)

The no-observed-adverse-effect-level (NOAEL) of Bt brinjal in Sprague Dawley rats following oral administration for 90 days was found to be more than 1000 mg/kg body weight.

8. Assessment of allergenicity using Brown Norway Rats (Rallis, Bangalore)

It is concluded that there is no biological difference between the allergenicity of the Bt and non-Bt brinjal

9. Primary skin irritation test in rabbits (Intox, Pune)

Based on the irritancy index, the Bt brinjal is to be classified as non-irritant to rabbit skin



10. Mucous membrane irritation test in female rabbit (Intox, Pune)

Based on the average irritation index, the Bt brinjal can be classified as non-irritant to mucous membrane in rabbits.

11. Subchronic feeding study in New Zealand White Rabbits (Advinus Therapeutic, Bangalore)

Based on the health, growth and physio-pathological parameters analyzed there is no differences between the transgenic Bt brinjal and control non- Bt brinjal fruit fed groups.

12. Feeding studies with common carp (fish) (Central Institute of Fisheries Education, Mumbai)

On the basis of isocaloric and isoproteinaceous feed the fish growth responses and histopathological alterations in gill, liver, intestine and kidney tissues was similar.



13. Feeding studies with broiler chicken (Central Avian Research Institute, Izatnagar)

Brinjal is a moderate energy rich feedstuff and can be safely incorporated up to 10% level in maize-soy based broiler rotation

14. Subchronic feeding study in goats (Advinus Therapeutic, Bangalore)

There are no differences between the Bt brinjal and non- Bt brinjal fruit fed groups based on the health, growth and physio-pathological parameters analyzed.

15. Feeding studies in lactating crossbred dairy cows

(G.B.Pant University of Agriculture and Technology, Pantnagar)

No adverse affects were seen on the health of the lactating crossbred cows, fed with Bt and non-Bt Brinjal mixed feeds.



16. Effects on non-target organisms

Effects on soil microbiota

- ✓ Multiple years of study
- ✓ No differences were seen in
 - Cultivable bacterial and fungal populations
 - Collembola populations
 - Earthworm populations
 - Soil nematode populations
 - Cry1 Ac protein level in the soil - through insect bioassays

Effect of Bt brinjal on non-target arthropods

Observations of non-target pests and beneficial insects show no significant differences between the Bt hybrids, their non-Bt counterparts, local and commercial checks.

