

# 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

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- 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

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- 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

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## 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

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- 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

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- 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

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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



# 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

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### 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.

