

भारत सरकार  
विज्ञान और प्रौद्योगिकी मंत्रालय  
बायोटेक्नोलॉजी विभाग  
GOVERNMENT OF INDIA  
MINISTRY OF SCIENCE & TECHNOLOGY  
DEPARTMENT OF BIOTECHNOLOGY

ब्लॉक-2, 7 वां तल, सी० जी० ओ० कम्प्लेक्स  
लोदी रोड, नई दिल्ली-110003  
Block-2, (7th Floor) C.G.O. Complex  
Lodi Road, New Delhi-110003

**LIMITED TRIAL PERMIT**

No.BT/17/02/94-PID

Dated: 11.06.2004

To

M/s. Maharashtra Hybrid Seeds Company Ltd,  
Resham Bhawan, 4<sup>th</sup> Floor,  
78, Veer Nariman Road,  
Mumbai - 400 020.

Subject: Application submitted for permission to carry out multi-location contained limited field trials on transgenic brinjal (*Solanum melongena*) containing *cy1Ac* gene and their non-Bt counter parts for assessing environmental safety by M/s. Maharashtra Hybrid Seeds Company Ltd, Mumbai during Kharif 2004.

Gentlemen,

The Department is to refer to your letter No. SBD/190/2004 dated 07.05.2004 on the above mentioned subject and to inform you that your application was considered by the Review Committee on Genetic Manipulation (RCGM) in its meeting held on 13.05.2004. On the basis of the recommendations of the RCGM, the Department is permitting you to conduct multi-location contained limited field trials on transgenic brinjal (*Solanum melongena*) containing *cy1Ac* gene and their non-Bt counter parts for experimental purposes at 11 locations during Kharif-2004, **subject to the following objectives and conditions:**

(a) The trials would be conducted in 5 repeats with randomized block design (RBD) at **Ahmednagar, Solapur, Pune** (Maharashtra), **Dharamपुरी** (TN), **Tumkur, Dharwad** (Karnataka), **Jalandhar** (Punjab), **Mirzapur** (Haryana), **Bhopal** (MP), **Alwar** (Rajasthan) and **Kurnool** (AP). The 5 Bt brinjal hybrids to be evaluated in these trials are **MHB-4 Bt, MHB-9 Bt, MHB-10 Bt, MHB-80 Bt** and **MHB-99 Bt** along with their non-Bt. counterparts and local & popular hybrid checks. The objectives of the trials is to generation of data on the following:

- i) To evaluate the efficacy of Bt brinjal hybrids on infestation of shoot borer and fruit borer in open field conditions in comparison to non-Bt brinjal hybrids.
- ii) To assess the effect of Bt brinjal hybrids on non-target pests & beneficial insects.
- iii) To assess the agronomic performance of Bt brinjal hybrids in comparison to non-Bt hybrid brinjal.
- iv) Estimation of the levels of expression of Bt. protein in Bt brinjal hybrids fruits at different growth period on regular interval of 15 days. The expression of Bt. protein shall be in milligrams of Cry1Ac protein per gram fresh weight of the fruit.
- v) Generation of baseline susceptibility data on shoot and fruit borer (*Leucinodes orbonalis*) and fruit borer (*Helicoverpa armigera*) population collected from the site of trial and rearing them at the premises of the company's bio-assay laboratory at Mahyco Life Science Research Centre, Aurangabad-Jalna Road, Jalna.

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तार / Telegram : 'BIOTECH' दूरभाष / Telephone : 4363012, 4360899 टेलिक्स / Telex : 31-74105 BIOT IN  
फैक्स / Fax : 011-4362884

b) The field design of the trials at above-mentioned locations would be the same as was submitted to the Department through your application-dated 07.05.2004. The experimental plot would be measuring 669.60 sq. meters (37.2 x 18.0 meters). An isolation distance of 200 meters from the periphery of the nearest row of transgenic brinjal has to be maintained where you are required to plant at least 50 rows of the non-Bt counterparts to act as the pollen trapper rows all around the experimental plot to cover the distance of 50 meters. You shall not plant any sexually compatible plants to the brinjal in the remaining isolation distance of 150 meters. The experimental layout is annexed at **Annexure-1**. You would be submitting the details of the locations to the Department before undertaking experiments.

c) You would mount a Notice Board at the site of experiment indicating in the Notice Board the purpose and duration of the experiment as well as the authorization under which the experiments are being conducted.

d) You would provide with three photographs of experimental site, taken from a distance sufficient to indicate the transgenic plots in a single photograph; such photographs would be taken at three intervals during the season to document the start of the experiments, the mid way of the experiments and the end of the experiments. These photographs would be submitted to the Government at the conclusion of the experiments.

e) You would keep full account of the transgenic materials and seeds, if any, set in the transgenic plants. All materials after experimentation including the seeds of cotton for the trapper rows would be fully accounted for and the information would be documented and preserved in a bound book that would be available to the Government as when requested for. The harvested crop from the border rows and leftover plant and plant parts from entire experimental plot shall be destroyed by burning after completion of the experiment.

f) You would collect and provide all the experimental data to the Government for inspection whenever required.

g) You would ensure that while performing the experiments, the "Recombinant DNA Safety Guidelines-1990" and "Revised Guidelines for Research in transgenic plants & Toxicity and Allergenicity evaluation of transgenic seeds, plants and plant parts" of the Government of India would be strictly adhered to. Accidents, if any, arising out of the experiments may be brought to the notice of the Government immediately.

h) You would further ensure that only company authorized personnel would be permitted to visit the experimental plot and persons visiting the experimental plot shall enter the name, designation and purpose of visiting the experimental plot in a bound book which should be made available to the Government when requested for.

i) You would extend full cooperation to the authorized personnel of the RCGM/MEC as well as the State Government Officials or their nominee to inspect the experimental sites and to have **access, for official use only**, the experimental results of the above.

j) You would inform the concerned State Government Officials about the conduct of the experiments in their states.

2. The Limited Trial Permit is valid for **Kharif Season 2004** only from the date of issue and would lapse automatically after the season.

Please acknowledge the receipt of this letter.

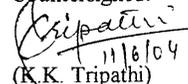
Yours faithfully,



(T.V. Ramanaiah)

Member-Secretary, RCGM &  
Scientist-F, DBT

Countersigned:



(K.K. Tripathi)

11/6/04  
Scientist-G, DBT

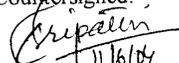
Copy for information and necessary action wherever applicable to:

1. The Secretary, Ministry of Environment and Forests, Paryavaran Bhawan, CGO Complex, Lodi Road, New Delhi - 3.
2. The Secretary, Ministry of Agriculture, Krishi Bhawan, New Delhi -110 001.
3. The Chairman, GEAC, MoE&F, Paryavaran Bhawan, C.G.O. Complex, New Delhi-110 003.
4. The Director General, ICAR, Krishi Bhawan, New Delhi -110 001.
5. The Chairman, MEC & Director, IARI, Pusa, New Delhi - 110 012.
6. The Chief Secretary, Government of Maharashtra, Mumbai.
7. The Chief Secretary, Government of Tamil Nadu, Chennai.
8. The Chief Secretary, Government of Karnataka, Bangalore.
9. The Chief Secretary, Government of Punjab, Chandigarh.
10. The Chief Secretary, Government of Haryana, Chandigarh.
11. The Chief Secretary, Government of Madhya Pradesh, Bhopal.
12. The Chief Secretary, Government of Rajasthan, Jaipur.
13. The Chief Secretary, Government of Andhra Pradesh, Hyderabad.
14. The Commissioner Agriculture, Maharashtra State, Central Building, Pune (Maharashtra).
15. The Commissioner of Agriculture, Department of Agriculture, Cheupak, Chennai.
16. Agriculture Commissioner, Department of Agriculture, Punjab State, Chandigarh.
17. Agriculture Commissioner, Department of Agriculture, Haryana State, Chandigarh.
18. Director of Agriculture, Rajasthan State, Jaipur.

Contd..4/-

19. The Commissioner of Agriculture, Department of Agriculture, Vidhyachal Bhawan, Near Secretariat, Bhopal (MP).
20. The Commissioner of Agriculture, Directorate of Agriculture, Sheshadri Road, Bangalore.
21. The Commissioner of Agriculture, Department of Agriculture, Hyderabad., Karnataka.
22. File No. BT/BS/17/02/94-PID

  
(T.V. Ramanaiah)  
Scientist-F, DBT

Countersigned:  
  
(K.K. Tripathi)  
Scientist-G, DBT

ANNEXURE -1

**Protocol for Replicated Research Field Trials of Bt Brinjal Hybrids containing *cry1Ac* gene**

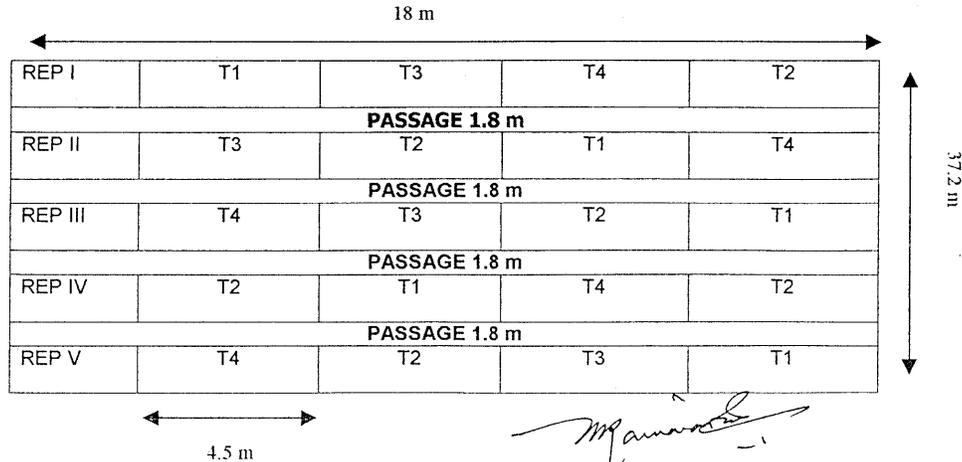
**Experimental Details :**

Number of trials : 11  
 Experimental Design : RBD  
 Treatments : Four

- Trial – I T1-MHB-4 (Bt), T2-MHB-4 (Non Bt),  
 T3-Local check, T4-Ajay Hybrid (Ankur seeds)  
**Location – Ahmednagar, Solapur (Maharashtra)**
- Trial – II T1-MHB-9 (Bt), T2-MHB-9 (Non Bt)  
 T3-Local check, T4-Extra Green Long Hybrid (sungro seeds)  
**Location – Dharmapuri (Tamilnadu), Tumkur (Karnataka)**
- Trial – III T1-MHB-10 (Bt), T2-MHB-10 (Non Bt),  
 T3-Local check, T4-Manju Hybrid (Syngenta seeds)  
**Location – Pune (Maharashtra), Dharwad (Karnataka)**
- Trial – IV T1-MHB-80 (Bt), T2-MHB-80 (Non Bt)  
 T3-Local check, T4-Navkiran Hybrid (Sungro seeds)  
**Location – Jalandhar (Punjab), Mirzapur (Haryana),  
 Bhopal (Madhya Pradesh), Alwar (Rajasthan)**
- Trial – V T1-MHB-99 (Bt), T2-MHB-99 (Non Bt),  
 T3-Local check, T4-Harit hybrid (Syngenta seeds)  
**Location – Karnool (Andhra Pradesh)**

Replications : 5; Row per plot : 5; Row length : 6 m; Plants per treatment : 50;  
 Spacing between rows : 90 cm; Spacing between plants : 60 cm; Space between replications :  
 1.8 m; Gross experimental plot area : 669.60 Sq.m; Net plot experimental area : 540 Sq.m; Net  
 plot per treatment : 16.2.Sq.m; Net area under Bt Brinjal hybrids : 81 Sq.m. (16.2 Sq.m x 5)

**Field Layout of Replicated Research Trial : (18m x 37.2m)**



**Dr. T.V. RAMANAIAH, M.Sc., Ph.D.**  
 Scientist-F  
 Department of Biotechnology  
 Ministry of Science & Technology  
 Block 2, 6-8, Floors, C.G.O. Complex,  
 Lodhi Road, New Delhi-110 003

## Farmers and Locations Details

State	District	Village	Farmers Name
Maharashtra	Pune	Shiroli	Mr. Kanifnath Narayan Sawant
Maharashtra	Ahmednagar	Rayate	Mr. Kisan baburao Kharde
Maharashtra	Solapur	Puluj	Mr. Mahadev Vasudeo Mamhane
Karnataka	Dharwad	Agadi	Mr. Nagappa T. Hangal
Karnataka	Tumkur	Gubbi	Mr. Vijay Kumar
Tamil Nadu	Dharmapuri	Basipatti	Mr. Selvaraj
Andhra Pradesh	Karnool	Pandipadu	Mr. B. Ramanjaneyalu
Haryana	Hissar	Mirzapur	Mr. Hamarayan Singh
Punjab	Jalandhar	Nanaksar	Mr. S.H. Baljindra Singh
Madhya Pradesh	Bhopal	Binapur	Mr. Torasingh Prajapati
Rajasthan	Alwar	Livari	Mr. Kesav Prasad Choudhary

## Summary Results

Replicated research trials of Bt brinjal hybrids for different market segments of India were approved by RCGM in 2004. Bt brinjal technology represents a new approach of transgenic resistance against Brinjal Fruit and Shoot Borer (BFSB). Primary objectives of these trials were as follows: (1) to assess the yield potential of the Bt brinjal hybrids, as compared to non-Bt brinjal hybrids commonly cultivated; (2) to compare lepidopteran pest (BFSB) load and feeding damage, between Bt brinjal hybrids and non- Bt hybrids; and (3) to study possible effects of the Bt gene against secondary lepidopteran pests and non-target insects.

Five Bt brinjal hybrids and three checks were grown in completely randomized block designs with five replications, at eleven locations. Trials of MHB-4Bt were conducted at two locations in Maharashtra, MHB-9Bt were conducted at locations in Tamil Nadu and Karnataka, MHB-10Bt were conducted at a location in Maharashtra and Karnataka, MHB-80Bt at Punjab and Madhya Pradesh locations and MHB-99Bt at one location in Andhra Pradesh. Check entries for all trial sets included Bt-hybrid, the non-Bt hybrid counterpart, local check and a commercial check. Data were collected on eggplant fruit and shoot borer infestation and related damage to plant parts, fruit count and brinjal yield over pickings. Supplemental information was collected on secondary lepidopteran pests, sucking pests, and beneficial insects.

Results from these experiments indicated that Bt brinjal hybrids under trial had potential for higher marketable brinjal yield in comparison to the non-Bt counterparts and checks grown in the area. All Bt hybrids had significantly higher marketable brinjal yield compared to non-Bt hybrid checks, at all trial locations. All Bt hybrids also had significantly higher fruit counts as compared to all three checks. Marketable fruit yield is a measure of quality. All Bt hybrids had greater number of marketable fruits than the non-bt hybrid check.

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 (*cryIAc*) and all three non-Bt checks. All Bt

hybrids were significantly lower in number of BFSB observed. Differences were also measured between the Bt hybrid group and check hybrids for shoot damage to plant from BFSB infestation. Percentage damage to shoots and fruits 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.

Supplemental observations were taken up to 150 days after transplanting for the presence of secondary lepidopteran pests, sucking pests and beneficial insects. For the brinjal fruit borer (*Helicoverpa armigera*), infestation was negligible in all treatments at all locations. For the presence of sucking pests (aphids, jassids, whitefly) and beneficial insects (*Chrysopa*, lady-bird beetle, spiders), no significant differences were noted between Bt and check hybrids.

Results of these multi-location replicated research trials indicate that the Bt brinjal hybrid entries are suitable for cultivation in India; from the standpoint of yield potential, BFSB efficacy and marketable yield. Bt brinjal hybrids showed a tendency for lower damage resulting from eggplant fruit and shoot borer feeding in comparison to non-Bt brinjal; as well as strong efficacy against eggplant fruit and shoot borer in comparison to non-Bt hybrids. Bt hybrid brinjal does not appear to have detrimental effects on non-target insects, including beneficial insects; and therefore can play a positive role within integrated pest management strategies for sustainable brinjal cultivation.