

Protocol for the Hybrid Seed Production of Bt Brinjal Hybrids MHB-11 Bt and MHB-39 Bt Containing the *cryIAc* Gene During Kharif 2006

Objective

The proposed hybrid brinjal seed production is for making commercial seed available upon approval by respective government authorities.

Experimental seed production design

Transgenic hybrid seed production of the hybrids containing *cryIAc* gene will be organized in Kharif-2006 season in the states of Maharashtra and Karnataka. The hybridization for seed parent will be carried out by crossing the female parent with the male parent.

Field layout of transgenic brinjal hybrid seed production

<p>(A)</p> <p>Female or Seed Parent Zone</p>	<p>N O P L A N T I N G</p>	<p>(B)</p> <p>Male Parent Zone</p>
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Isolation

For hybrid seed production, isolation from other brinjal crops including seed production of other varieties or hybrids shall be maintained.

Ratio of male and female parent

The planting area of female and male parent shall be in the ratio of 3:1 with a no planting zone of two meter as a demarcation between the two zones of seed (Female) parent and pollinator (Male) parent.

Planting distances

Male: Spacing between row-to-row shall be 90cm and plant-to-plant 60cm .

Female: Spacing between rows to row shall be 90 cm and plant-to-plant 90cm.

Hybridization

On the female parent mature buds are emasculated in the afternoon and covered with colored paper packets to avoid any contamination from foreign pollen. Third day morning anthers of male buds/ freshly opened male flowers are removed and kept under sunlight/ burning electric bulb at a slightly higher temperature which helps in bursting of anther wall and exposing the pollen which in turn is separated by special mechanism of filtration using nylon plastic fine mesh. The pollen grains in the form of powder are filled in small plastic tube attached to a plastic ring that is used for the pollination work. The pollinated buds are tied with a thread and covered with white packets.

Removal of male parent

On completion of pollination work during the season the pollinator parent will be uprooted and incinerated. No fruit formation will be allowed on this parent at any stage.

Seed extraction

Harvesting is done when fruits are fully ripe. The tagged fruits are picked and collected. The outer covering is peeled off and the flesh with the seed is cut in to thin slices. These are then softened by soaking in water and seeds are extracted manually. The

extracted seed is cleaned in water repeatedly to separate all the light immature seeds and dried in partial shade to a moisture content of 8% or below before storing.

Processing and cleaning

After receiving the unprocessed seed at processing plant, seed will be processed and cleaned. Sample will be drawn for testing the genetic purity, germination, physical purity and Bt trait purity of the seed lot. All remnant seed shall be incinerated. The cleaned seed shall be stored in a secured place and shall be used as and when permission is received from the regulatory authority. Accounts of all quantities shall be maintained and will be produced for inspection on demand by the authorities.

The Company also undertake that it will not supply/ sell/ use the seeds of any hybrid/s till it is/ they are permitted/ released by GEAC for the purpose.

Name of the transgenic brinjal hybrids

The following transgenic insect resistant Bt brinjal hybrids containing *cryIAC* gene will be produced in the Kharif-2006 season.

Hybrids for Seed Production	Area of Seed Production
MHB-11 Bt	4 ha.
MHB-39 Bt	4 ha.

Locations

The hybrid seed production will be organized on company's own/ leased farms or on farmer's fields in the states of Maharashtra and Karnataka under the direct control and supervision by the technical staff of Mahyco.