

Frequently Asked Questions (FAQs)

What is this application related to GM Mustard?

This application relates to Environmental release of Genetically Engineered Mustard (*Brassica juncea*) hybrid DMH-11 and use of parental events (Varuna bn3.6 and EH2 modbs2.99) for development of new generation hybrids

Who is the developer of this technology?

Centre for Genetic Manipulation of Crop Plants (CGMCP), University of Delhi, South Campus, New Delhi has developed this Barnase-Barstar technology

Was this project funded by Government of India?

Yes, this project received financial support from Department of Biotechnology (DBT) and the National Dairy Development Board (NDDB)

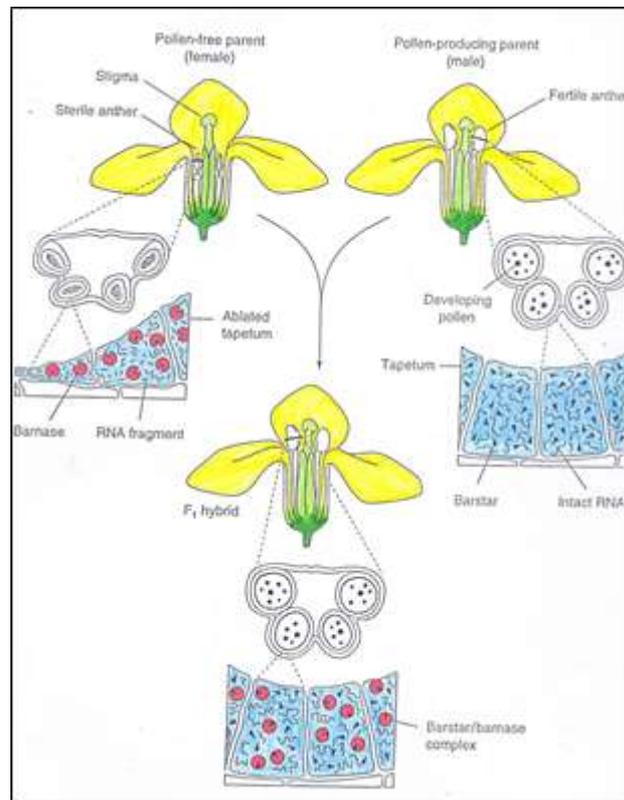
What is this technology about?

Mustard flowers contain both male and female organs and the crop is pre-dominantly self-pollinating. Therefore, a pollination control mechanism is required to disallow self-pollination and encourage cross-pollination for hybrid seed production. For this, one of the two parental lines of a hybrid has to be made male sterile so that it receives pollen from the other parent to set seed. Seeds harvested from the male sterile line are hybrid seeds which can be provided to the farmers, who can reap the benefit of higher productivity of the hybrids.

Male sterile lines can be developed by using cytoplasmic male sterility (CMS) through conventional breeding or by genetic engineering using transgenes. A number of CMS systems have been tested in mustard. However, CMS/restorer systems have been found to be inadequate for large scale hybrid seed production with high purity. CMS systems are either unstable or their restoration to fertility is inadequate. A more versatile hybrid seed production system is based on the use of transgenes – *barnase* and *barstar* as explained below.

A novel way to developing male sterile (MS) lines through genetic engineering was developed by scientists in Belgium in early 1990s through the use of two genes – *barnase* and *barstar* from soil bacterium *Bacillus amyloliquefaciens*. In nature, bacterium excretes a defense protein called Barnase (a type of ribonuclease) which degrades the RNA of competing bacteria in an ecological niche. To protect itself from Barnase, the bacterium produces another protein called Barstar which tightly binds with Barnase and renders it ineffective. Bacterial genes can only express in plants if these are expressed under plant promoters. Both Barnase and Barstar encoding genes were expressed under a tapetum specific promoter. Tapetum is a layer of cells in the male organs called anthers present in the flower. Tapetum produces metabolites which are essential for the development of mature pollen. In the *barnase* gene containing lines, the tapetum tissue ablates (dies) as a consequence developing pollen degenerate, providing MS lines. The other parental line, called restorer of fertility (RF) line, contains

barstar gene that also expresses in the tapetum cells. The MS line receives pollen from the RF line through wind pollination or bee pollination, resulting in the production of hybrid seed that has both the *barnase* and the *barstar* genes. When hybrids are grown by the farmer these are fully fertile. Thus the MS/ RF system ensures that the MS line will only produce hybrid seeds by outcrossing with RF lines thereby providing an efficient system of pollination control for production of hybrid seed. The system hereafter is referred to as *barnase-barstar* system.



What are the issues of concern for the environment?

Issues of concern include the capability of the GE Plant to escape and potentially introduce the engineered genes into wild populations; the persistence of the gene after the GE Plant has been harvested; the susceptibility of non-target organisms (e.g. insects which are not pests) to the gene product; the stability of the gene; the reduction in the spectrum of other plants including loss of biodiversity; and increased use of chemicals in agriculture.

What safety studies were done for ensuring that GM Mustard is safe and were they done according to relevant guidelines

The following safety studies were done for GM Mustard and they were as per prescribed guidelines developed by Government of India from time to time

Category	Tests Done	Relevant Guidelines
Molecular characterization	<ul style="list-style-type: none"> • Gene sequences, constructs and molecular characterization • Expression studies of the three inserted genes – <i>bar</i>, <i>barnase</i> and <i>barstar</i> • Cloning, expression, purification and production of three expressed proteins 	<ul style="list-style-type: none"> • Recombinant DNA Safety Guidelines, 1990 • Guidelines for the Safety Assessment of Foods Derived from Genetically Engineered Plants, 2008 prepared by Indian Council of Medical Research (ICMR), Ministry of Health and Family Welfare, Government of India
Food safety Studies	<ul style="list-style-type: none"> • Equivalence of the Bar, Barnase and Barstar recombinant proteins produced in bacteria with that expressed in GE plants • Bioinformatics analysis of the three proteins • Pepsin digestibility of the three proteins • Heat stability of the three proteins • Acute oral toxicity of the three proteins in mice • Sub-chronic toxicity of leaves and seeds containing the three proteins in rats • Compositional analysis 	<ul style="list-style-type: none"> • Guidelines for the Safety Assessment of Foods Derived from Genetically Engineered Plants, 2008 prepared by Indian Council of Medical Research (ICMR), Ministry of Health and Family Welfare, Government of India
Environmental safety studies	<ul style="list-style-type: none"> • Research & Development Phase including primary field trials 2004-2007. • BRLI trials for two growing seasons (2010-11, 2011-12) • BRLII trials for one growing season (2014-15) • Weediness potential and aggressiveness parameters 	<ul style="list-style-type: none"> • Revised Guidelines for Research in Transgenic Plants, 1998 (DBT) • Guidelines for the monitoring of Confined Field Trials of Regulated, Genetically Engineered (GE) Plants, 2008 (DBT & MoEF&CC)

	<ul style="list-style-type: none"> • Impact on soil microflora during BRLI and BRLII trials • Crossability and pollen flow studies • Pollination behavior, pollen morphology and physiology 	
Detection Protocols	<ul style="list-style-type: none"> • Protocol for testing at a level of detection (LOD) of 0.01% • Development of ELISA kits for Bar, Barnase and Barstar 	<ul style="list-style-type: none"> • Tests and Detection methods developed

Were any Public Sector Institutions engaged in conducting safety studies related to this technology?

Yes, the following Public Sector Institutions were engaged in conducting safety studies related to this technology

- ICAR-Directorate of Rapeseed Mustard Research (DRMR), Bharatpur was engaged for conduct and monitoring of confined field trials (CFTs) and generation of data related to environmental safety studies
- ICMR-National Institute of Nutrition (NIN), Hyderabad was engaged for undertaking all food safety related studies
- CSIR-Institute of Microbial Technology (IMTECH), Chandigarh was engaged in conduct of soil micro flora studies.

Are GM foods safe?

Different GM organisms include different genes inserted in different ways. This means that individual GM foods and their safety should be assessed on a case-by-case basis and that it is not possible to make general statements on the safety of all GM foods. GM foods currently available on the international market have passed safety assessments and are not likely to present risks for human health. In addition, no effects on human health have been shown as a result of the consumption of such foods by the general population in the countries where they have been approved.

What kind of GM foods are on the market internationally?

GM crops available on the international market today have been designed using one of the following basic traits: resistance to insect damage; resistance to viral infections; and tolerance towards certain herbicides. GM crops for hybrid seed production for heterosis breeding (e.g. rapeseed/canola) and with higher nutrient content (e.g. soybeans increased oleic acid) have been also studied recently.

Will the transgene get transferred to humans or animals when GM Food is consumed?

The transgenes would not get transferred to humans or animals through consumption of GE mustard. So far, there is no evidence suggesting that the transgenes could be transferred to humans or animals through consumption of GE food.

Will the introduced proteins in GE mustard have toxicity?

The regulatory agencies assessed thoroughly the dossier and evaluated critically the safety data on toxicity and allergenicity in humans and animals and found that the introduced proteins do not have any toxicity.

Can the seeds of this GE mustard be directly consumed?

The seeds of this GE mustard are as safe for consumption as other conventionally grown mustard varieties in India.

Will it be safe to consume leaves of this GE mustard?

This GE mustard leaves are as safe for consumption as other conventionally grown mustard varieties in India.

Will medicinal products containing mustard oil or leaf from this GE mustard hybrid be safe?

Medicinal products containing mustard oil or leaf from this hybrid have been assessed to be as safe as other conventional mustard varieties.

Will my genome structure be altered by consuming this GE mustard? or Will GE mustard make me a monster?

The introduced Barnase and Barstar proteins of the GE mustard are expressed in specific tissue (anther) and are not found in other parts of the plant. Bar protein expressed in leaves but is rapidly degraded during cooking. Also, the expressed proteins show rapid digestion in the human gut in the in vitro tests. Moreover there has been more than a decade of history of safe use of these three proteins hence it can be concluded that these proteins would not alter anyone's genome after consumption of this GE mustard.

Will this GE mustard taste different?

The compositional analysis of this GE mustard shows that all the biochemical/nutritional components of GE mustard are similar to that of conventionally grown mustard varieties (varuna and RL1359). Therefore, GE mustard is not expected to have a change in the taste.

Will GE mustard be more pungent?

The compositional analysis of GE mustard reveal that Glucosinolates content (responsible for pungency) in this GE mustard is similar to commercially grown variety in India (Varuna and RL1359). So, this GE mustard is not expected to have a change in the taste.

Will the male sterility-fertility restorer technology has impact on humans?

The genes responsible for male sterility-fertility restorer technology are specific to plant cells only and have no selectivity to human reproductive cells. Moreover, in the 90-day sub-chronic feeding studies conducted with edible plant parts (leaf and seed) no toxic effects were observed on reproductive organs of rats.

Is this GE mustard still nutritious?

In compositional analysis, this GE mustard is found to be a substantially equivalent to commercially cultivated mustard variety Varuna and RL1359. Hence, GE mustard would be as nutritious as the conventionally grown varieties in India.

Is this GE mustard a high protein diet because of introduced protein?

The introduced proteins are expressed in low to negligible level in the edible parts (leaf and seed) of this GE mustard. Hence, this GE mustard is as nutritious as other conventional varieties.

Will cattle produce more milk if this GE mustard is used as feed?

This GE mustard is as nutritious as the conventionally grown varieties in India. Therefore, it is not anticipated to have any effect on milk production, if cattle consume this GE mustard as feed.

Will milk produced by cattle having this GE mustard in diet be different in colour and other nutritional value?

In compositional analysis, this GE mustard is found to be substantially equivalent to commercially cultivated mustard varieties (Varuna and RL1359). Hence, this GE mustard will have same nutritional value. Therefore, milk produced by cows after the consumption of this GE mustard, if any, will not change in terms of colour and nutritional value.

Will mixing of this GE mustard oil with other oil result in dietary toxicity?

Oil from this GE Mustard contained no new proteins. Therefore, this GE mustard oil is as safe as the conventional mustard oil from the non-transgenic mustard variety.

After inhalation of this GE mustard pollen, will it have any effect on our body?

Mustard crop have been cultivated for a long time in India. Pollen characteristics of this GE mustard have been assessed and found to have no abnormal characters as compared to conventionally grown varieties.

Will oil derived from GE mustard be safe for dermal and cosmetic use?

Composition of oil from this GE mustard is similar to oil derived from conventional mustard variety and therefore, the oil derived from this GE mustard is as safe as the oil from conventional varieties. Hence, people allergic to traditional mustard oil should exercise similar caution to this GE mustard.

Will animals grazing on this GE mustard be safe?

The three introduced proteins (Barnase, Barstar and Bar) are expressed in very less amount in the plant and are found to be non-toxic in the acute as well as sub-chronic toxicity studies in rats and mice. Based on the Global reports of history of safe use and other reported scientific literature, it is assessed to be safe for animal grazing on it.

Will the GE mustard cake be safe for animal feed?

All the three introduced proteins as well as studies using edible plant parts did not show any adverse effects. Moreover, seed and meal from transgenic canola having same barnase-barstar system is being fed to animals in various countries and no adverse effects have been reported so far. Thus, this GE mustard cake is assessed as safe for feeding animals.

Will it alter beneficial gut microbial flora in human and animal?

The assessment for the effect of this GE mustard on microbial population has revealed no adverse effect on soil microorganisms. Also, the three introduced proteins have been found to be easily digested by the simulated gastric fluid. Therefore, based on the history of safe use of these proteins in other countries, it can be concluded that this GE mustard would not alter gut microbial flora in human and animals.

Will expressed protein leach to water table making it toxic for consumption?

The Barnase, barstar and bar proteins are produced by naturally occurring soil microorganism. There are no such evidence of leachates of these protein in ground water. Further, it has to be noted that these proteins are not stable for a long period and gets degraded after release in the soil.

Whether GE mustard will cause Dropsy effect?

This GE mustard is as safe as non-GE mustard. It won't cause Dropsy or any other diseases.

Will GE mustard affect quality of nectar?

The introduced proteins are independent of the nectar production traits and therefore are not expected to influence nectar quality and quantity.

Will the number of honey bees visiting this GE mustard fields are less as compared to non-GE mustard fields?

The honey bee studies have revealed that this GE mustard does not affect the number of honey bees visiting GE mustard plants as compared to non-GE mustard plants.

Will GE mustard require any insecticidal sprays?

GE mustard requires no additional care for growth and cultivation as compared to any other conventional mustard.

In what season can it be grown?

Mustard is grown as a Rabi crop in India. All the agronomic practices are same as it is in the conventional mustard.

Whether farmers are required to use herbicides for cultivation of GE hybrid Mustard?

No herbicide is required or recommended for cultivation of GE hybrid Mustard.

What will be the additional irrigation and fertilizer requirements of this GE mustard?

GE mustard has no additional requirement of irrigation and fertilizer for growth and cultivation as compared to any other conventional mustard.

Will this GE mustard be insect resistant like Bt cotton?

This GE mustard has no insecticidal proteins, hence would behave similar to conventionally grown mustard.

Will there be any colour change in the GE mustard so that it can be identified separately?

The introduced proteins in this GE mustard has no role in colour determination, hence would be similar to conventionally grown mustard.

Can I grow GE mustard domestically?

GE mustard has no additional requirement for growth and cultivation as compared to any other conventional mustard. Hence, it can be grown domestically with similar cultivation practices.

Will this GE mustard hybrid produce fertile flower?

The hybrid GE mustard with Barnase-Barstar technology will lead to production of fully fertile flowers.

Will this GE mustard hybrid produce sterile seed?

The hybrid GE mustard with Barnase-Barstar technology will lead to production of fully fertile seeds in the farmer's field.

Does this GE mustard hybrid has the terminator technology?

This GE mustard hybrid is developed using Barnase-Barstar technology and do not has the terminator technology.
