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Dhara Mustard Hybrid-11

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Dhara Mustard Hybrid-11 is a genetically modified hybrid variety of the mustard species Brassica juncea. It is a genetically modified variant of Herbicide Tolerant (HT) mustard. DMH-11 is a result of a cross between Indian mustard variety 'Varuna' and East European 'Early Heera-2' mustard. It contains two alien genes ('barnase' and 'barstar') isolated from a soil bacterium called *Bacillus amyloliquefaciens* that enable breeding of high-yielding commercial mustard hybrids. Barnase in Varuna induces a temporary sterility because of which it can't naturally self-pollinate. Barstar in Heera blocks the effect of barnase allowing seeds to be produced. DMH-11 has shown approximately 28% more yield than the national check and 37 % more than the zonal checks and its use has been claimed and approved by the Genetic Engineering Appraisal Committee. "Bar gene" maintains the genetic purity of hybrid seed.

Keywords: Mustard, Barnse, Barstar, *Bacillus amyloliquefaciens*, Hybrid

Development procedure and Mechanism

The transgenic mustard DMH - 11 was developed in 2002 using genetic material isolated from non-pathogenic soil bacteria, and techniques in transgenic systems for pollination control, which primarily involved the Barnase-Barstar, system. Three genes, Bar, Barnase and Barstar, were extracted from *Bacillus amyloliquefaciens* to produce the hybrid seed.

Bacillus amyloliquefaciens is a species of bacterium in the genus *Bacillus* that is the source of the BamHI restriction enzyme. It also synthesizes a natural antibiotic protein barnse, a widely studied ribonuclease that forms a famously tight complex with its intracellular inhibitor barstar, and plantazolicin, an antibiotic with selective activity against Bacillus anthracis.

It is used in agriculture, aquaculture, and hydroponics to fight root pathogens such as *Ralstonia solanacearum*, *Pythium*, *Rhizoctonia solani*, *Alternaria tenuissima* and *Fusarium* as well improve root tolerance to salt stress. They are considered a growth-promoting rhizobacteria and have the ability to quickly colonize roots.

Barnase ("Bacterial" "RiboNucleASE") is a bacterial protein that consists of 110 amino acids and has ribonuclease activity. It is synthesized and secreted by the bacterium *Bacillus amyloliquefaciens***,** but is lethal to the cell when expressed without its inhibitor barster. The inhibitor binds to and occludes the ribonuclease active site, preventing barnase from damaging the cell's RNA after it has been synthesized but before it has been secreted. The barnase/barstar complex is noted for its extraordinarily tight protein - protein binding, with an on-rate of $10^8 s^{-1} M^{-1}$.

Barstar is a small protein synthesized by the bacterium *Bacillus amyloliquefaciens*. Its function is to inhibit the ribonuclease activity of its binding partner barnse, with which it forms an extraordinarily tightly bound complex within the cell

until barnase is secreted. Expression of barstar is necessary to counter the lethal effect of expressed active barnase. The structure of the barnase-barstar complex is known

The main reason for introducing the Barnase-Barstar gene system into the transgenic mustard line was for heterosis breeding and to prevent self-fertilization.

Heterosis, hybrid vigor, or outbreeding enhancement is the improved or increased function of any biological quality in a hybrid offspring. An offspring is heterotic if its traits are enhanced as a result of mixing the genetic contributions of its parents. The heterotic offspring often has traits that are more than the simple addition of the parents' traits, and can be explained by Mendelian or non-Mendelian inheritance. Typical heterotic/hybrid traits of interest in agriculture are higher yield, quicker maturity, stability, drought tolerance etc.

The insertion of the Barnase gene induces genetic male sterility by preventing the production of the male gametophytes (pollen grains) in the mustard plant. Meanwhile, the Barstar gene acts to restore the ability of the plant to produce fertile hybrid seeds. Mustard is a self-pollinating plant, thus, making it difficult to perform cross-pollination with another desired male parental line, without the occurrence of self-pollination. The Barnase gene induced male sterility in DMH - 11, simplifying the process of cross pollination to derive new hybrid varieties. The two parental strains used to develop DMH -11 are the Early Hira-2 and the Varuna. The seed weight of DHM-11 is reported to be around 3.3 to 3.5 grams (/1000 seeds.

DMH - 11's Glufosinate resistance is due to an enzyme expressed by the Bar (Bialaphos resistance) gene. Derived from *Streptomyces hygroscopicus*, the cloned Bar gene in DMH-11 encodes for the synthesis of Phosphinothricin acetyl-transferase (PAT).This enzyme is responsible for detoxifying the active ingredient in the herbicide Glufosinate: phosphinothricin. Phosphinothricin's mechanism of action involves the inhibition of Glutamine synthetase, which prevents the detoxification of ammonia and subsequently causes toxic buildup within plant cells.

This prevents the detoxification of ammonia and subsequently causes toxic buildup within plant cells. Inhibition of glutamine synthetase also leads to an overall reduction in Glutamine levels. In plants, Glutamine acts as a signalling molecule, and as a major amino acid donor for nucleotide synthesis. PAT enzymes produced by the Bar gene, deactivate Bialaphos (the tripeptide precursor to phosphinothricin) through acetylation to form an inactive, non-toxic product.

Major issues

Health: The Honorable Supreme Court of India appointed a Technical Expert Committee which in its 2013 report recommended a total ban on herbicide-tolerant transgenic crops. One of the reasons the TEC cited was the possibility of ensuing negative health effects as a result of farmers generously applying herbicide to their crops.

Ecological: DMH - 11 being commercially cultivated is the potential genetic pollution of the rich biodiversity of the genus Brassica, via cross pollination between DMH-11 and wild populations. Mustard plants undergo wind and insect mediated pollination. Due to its susceptibility to outcrossing, there is a possibility of India's rich mustard gerplasm consisting of wild varieties and domesticated landraces being irreversibly contaminated.

Social and economic: In 2004, the chairman of the Task Force in Agricultural Biotechnology advised against commercialisation of herbicide-resistant crops as they eliminate the need for manual weeding, a job undertaken by many rural women in India. It was estimated by Kavita Kuruganti, a member of the Alliance for Sustainable and Holistic Agriculture, that growing even 25% of DMH - 11 will result in a loss of 4.25 crore employment days for many women.

Yield: DMH - 11 demonstrated yield heterosis in the range of 19%–40% over some of the best Indian varieties. Another multi-site trial conducted under field conditions found the DMH - 11 to produce 30% more yield than existing strains of traditional mustard varieties.

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