Genetically Modified Mustard Plant
To become first modified food in India

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ABSTRACT
The review paper considers the future of Genetically Modified Plants. GM plants are attracting a large amount of media attention and is a major topic of debate owing to various positive and negative impacts of GM plants on the environment and the health of human beings. This paper discuss about the present scenario, techniques applied, procedure followed and future scenario of GM Mustard in India.

1. INTRODUCTION
GM crops have already widened their share in the agriculture market. Many countries have commercialized the food crops and have successfully included the GM crops in their meal. USA, being the first for commercialization of crops including both food and non–food crops. Many Asian and European country has passed the way for commercialization of various GM crops. Successful trials of GM crops and their effects on human health and environment have been conducted in the African countries. India is lagging behind owing slow pace of development of research center, scientific projects starve for the funding from the government, unwillingness by the farmers to go for a change and majorlry, more than expected hindrance is caused by opposition party by representing a galaxy of negative impacts due to GM crops. GEAC i.e. the government has taken a decision to allow commercial cultivation of Mustard DMH-11[1]. It is a transgenic crop declared by Dr. Pental and team at The Centre for Genetic Manipulation of Crop Plants at Delhi University. This move comes out after Bt Brinjal’s commercial approval has been hold since 2009. So far, India has officially approved only GM Cotton for commercial cultivation in the country.

2. Genetically Modified Plants
Genetically Modified Plants are developed to induce a new trait to the plant which does not occur naturally. DNA of the plant is modified using Genetic Engineering to produce GM plant varieties which can be grown as food crops or non-food crops. GM food crops passes the properties of resistance to certain pests, diseases or environmental factors. Many GM food crops have been developed which has higher level of nutrition value. GM non-food crops possess the ability to produce a variety of pharmaceuticals ingredients, biofuels and other industry oriented products. Various plant varieties have also been developed which is helpful in Bioremediation [2].

All the genetic information of an organism is stored in form of DNA molecules in chromosomes. The chemical base sequence of DNA strand encodes Amino Acids, which are the building blocks of proteins. Proteins are the major functional entity and carry out all the major function in cells or tissues which additively responsible for an organism’s characteristics. Science has gone so advanced in Molecular Biology & Genetic Engineering, various methods have been developed to make it possible to transfer a gene of interest from one species to another. The inserted DNA encodes specific protein, along with DNA sequence, such as promoter, enhancers, UCE which regulates the production of proteins. Marker gene is induced in the inserted fragment, for the easy identification of plants that incorporate the transferred gene [3].
3. History of GM Corn

In original Corn was domesticated in Mexico 10,000 years ago by the process of crosspollination. The process included transforming *teosinte*, a non-descript grass, into full cared Modern Corn. Biotech super giants such as Monsanto, Syngenta are manipulating the Corn genetics to satisfy farmer’s desires. Plant Breeding needs transformation and change for better therefore, Biotech super-giants are manipulating Corn genetics to satisfy farmer’s desire and the global market. Plant breeding was once only restricted to sexually compatible plants and generations of offsprings were selectively picked for breeding to create unique varieties. For e.g. - Herbicide resistance, Insects resistance, Disease resistance. Cytoplasmic Male sterility is in general natural mutation, which can also be induced chemically or manually. After realizing, that natural mutant introduce valuable traits, Scientists are turning to chemicals & Irradiation to speed the creation of mutants. According to APHIS, Corn is the second most common GM crop.

4. Advantageous Traits introduced to GM Corn

4.1 Insect Resistance

European corn borer causes most of the damage during larval stage. The Bt delta endotoxin is highly effective at controlling Lepidoptera larvae, caterpillars. Delta-endotoxin is very selective and does not harms other insects such as Beetles, Flies, Bees &Wasps and is safe for humans. Bt has been commercialized as an insecticide since 1960s, also possessing the insect killing ability. For commercialization, Bt yellow Maize was released in 1998. South Africa was 1st country to permit it for commercialization. Bt corn starch, along with GMO soy lecithin, were used in 70% of processed food in 2002[4]. The 1st generation of Bt crop has been extraordinarily success with few examples -

- StarLink –Cry9C Bt corn
- MON 802-an Insect Resistant maize developed by Monsanto Company. MON 802 was made tolerant to glyphosate herbicide & protect the plant from European corn borer, developed through genetic engineering.
- MON 863, MON 809, MON 810, expressing the Cry1Ac protein for Lepidopteran insects.
- MON- 877 1-2 – Resistance to Lepidopteran pests of soyabean including velvet bean caterpillar and soyabean looper have been developed using agrobacterium mediated plant transformation.

4.2 Disease Resistant

Corn is subject to plant diseases, including fungi & bacteria. GMO crops major benefit described in International Council for science, report cited by the “Public Library of Science –Biology “i.e. Bioengineered Corn ,carry disease resistance containing lower levels of mycotoxin. Mycotoxin is a substance produced by growing on insect plagued, non-GMO corn crops. Mycotoxins are carcinogenic, which can adversely affect human beings.

4.3 Herbicide Resistance

The approval for herbicides resistance crop came in 2002. Weed grow as a major competition to the main crop, in the main field, having no commercial or nutritive value. To get away from the weeds, herbicides are used to kill weeds but the herbicide can also damage agricultural products and the negative effects come along with the use of herbicides is the retention of herbicides in the food. GMO corn has been made resistant to the herbicides and pesticides. E.g.-DK 404 SR- cyclohexanedion herbicide tolerant GM-Maize
4.4 Increased Nutrition Value

Beta-carotene has been biofortified in Maize, having higher value of Beta-carotene than conventional white porridge Maize and this yellow Maize porridge provide 40-50% of vitamin A quantity recommended by U.S Dietary Allowance.

Vitamin fortified Maize has been developed through Metabolic Engineering which express high amount of beta-carotene, ascorbate & folate in its Endosperm.

4.5 Production of Pharmaceutical Ingredients

Important plant derived, recombinant products, avidin & Beta glucuronidase are produced from strains of GM corn. Genomic alterations of GM corn has been done to produce human aprotivin, protein useful in transplantation surgery. Trypsin, a pancreatic serine protease is produced by GM Maize. Dihydroxyacetone -A major ingredient in sunless tanning products are derived from GM corn. A Colorado trial of corn producing a protein to treat cystic fibrosis.

5. Genetically Modified Mustard in India

Pollen from one flower of a plant has to move to a flower of another plant to generate seeds, fruits and next batch of plants. But mustard is a self-pollinating plant. In other words, pollen need not move from one plant to another, but between parts of the same plant. In self-pollination, pollen from the anther (the male part of the plant) transfers to stigma (the female part of the plant) of the same flower or from another flower of the same plant making it fit for fertilization. To develop the mustard hybrid seed, one of the two parental lines had to be made male sterile – that is, the plant had to stop producing functional anthers, pollen, or male gametes – so that it would be forced to cross with the other line to produce a hybrid seed. Once the two parental lines were crossed, male sterility had to be restored in their hybrid progeny; otherwise no hybrid mustard seeds would be developed. Conventional hybridisation system produces perfect flowers, or flowers that have both male anthers and female pistils. Thus making it difficult for breeders to hand-pollinate one mustard variety with another to make hybrid. To overcome this problem without using transgenic, breeders used Cytoplasmic Male Sterility System, turning to a gene in wild mustard that impedes pollen formation. This gene, which occurs in the cell’s cytoplasm, is transferred to cultivated mustard by repeatedly crossing both plants. The cultivated mustard, known as Brassica juncea, now stops making pollen and can act as a female parent. Next, another gene from wild mustard, which can reverse the pollen sterility caused by the first gene, is transferred to another variety of Brassica juncea, which becomes the male parent. These two are then crossed to create a hybrid of both Brassica varieties. To add a trait to a crop plant, gene is inserted along the additional genetic material which helps in the expression of new trait in the plant. Marker genes help in the determination of expressed new trait in the plant. Herbicide & Antibiotic tolerant promoters are used for the identification of transformed plants.

The first of these hybrids, DMH-1 (a predecessor of DMH-11), was developed by Pental, he borrowed the male-sterility gene from a Brassica napus mustard variety. The gene was introduced into the female parent of DMH-11, an Indian Brassica juncea variety called Pusa Bold. Next, Pusa Bold was hybridised with an eastern European variety of mustard called EH-2, which is naturally able to reverse the sterility induced by the CMS gene. Pusa Bold and EH-2 made a good couple: when hybridised, they showed high heterosis and enhance the traits of their parents. As a result, DMH-1, the progeny of Pusa and EH-2, gives between 28% and 34% more yield than pure mustard varieties [5].
Drawbacks

DMH-1 could have been the hybrid Indian farmers were waiting for – but it also came with its flaws Pental’s team was unable to produce enough seed to grow this hybrid using CMS systems. Sterility often breaks down while growing the mustard for seed production, especially in frosty weather; the female parent pollinates itself, short-circuiting the hybridisation process. As a result, only around 85% of the seeds produced are pure hybrid seeds. Such a low level of seed purity is unusual for hybrids, with the department of agriculture typically requiring at least 95% purity.

The second problem with the CMS system was that it doesn’t work with a female parent other than Pusa Bold. “When it was tried with another female parent, other traits such as disease resistance, sterility breaked down again. This means that the possibility of improved hybrids with this CMS system was very much reduced.” Pental believed DMH-1 is as far as his cytoplasmic male-sterility system can go, and that was the time to try transgenic [6].

NRCHB506

The Indian Council of Agricultural Research has developed a hybrid called NRCHB 506 using the Moricandia system. Moricandia, borrows the sterility gene from a wild Brassica species called Moricandia arvensis. S.R. Bhat, plant biologist at the National Research Centre for Plant Biotechnology, who developed Moricandia says,” NRCHB works smoothly and can be used with any mustard variety, unlike Pental’s system”.

NRCHB isn’t popular in India because its yields are not as high as Pental’s DMH-1. Reason behind this is that NRCHB is a hybrid of two Indian varieties of mustard which do not show as much heterosis as the varieties Pental chose. This is where transgenics can play a role in speeding things up [7].

DMH-11

The transgenic hybrid developed by Pental, hybridises an Indian mustard variety called Varuna with the east European EH-2, and also involves transgenics to overcome the problems that plagued predecessor DMH-1. Pental says the new method is able to produce pure seed almost 99% of the time. This means the transgenic mustard can reach more farmers than its predecessor ever did. DMH-11 is based on Barnase- Barstar system. The Barnase gene codes for a protein that hinders the pollen production. The plant into which Barnase gene is introduced, made it male-sterile, and left it only capable of receiving pollen from another parent. Plant having fertile, potent male part contains the Barstar gene, which blocks the action of the Barnase gene. The resultant F1 progeny is both high yielding and is also potent to produce seed/grain, in the second male-fertile line. Bio-safety research level field trials done on DMH-11 has reported a roughly 30% more yield advantage over the Varuna ‘check’ variety carried out between 2010-11 and 2014-15 [8].

Barnase- Barstar system

Barnase is a bacterial protein consists of 110 amino acids and possess ribonuclease activity. Bacillus amyloliquefaciens synthesize and secretes the Barnase gene, which is lethal to the cell when expressed without its inhibitor barstar. The inhibitor binds to Barstar gene and impedes its ribonuclease active site, preventing cell’s RNA from damage by Barstar after it has been synthesized and before it has been secreted [9]. Bar gene is used as a marker to mark out the transgenic seeds having the desired characteristics after the hybrid crosses are done. The barnase and barstar genes don’t express in the seed, only the bar gene does, to some extent but it poses no threat [10].
Concerns Regarding GM Mustard

A group of scientists and environmental activists claim that there is an increasing concern about sustainable farming and low-input agriculture and to the surprise agricultural scientists are ending up recommending crop varieties that will end up doing more harm to the environment and crop fields and are only paying attention towards more yield. GM mustard would require almost twice the quantity of fertilizer and water [11].

• A lot Health concerns including allergenicity; gene transfer, especially of antibiotic-resistant genes arose after introduction of GM crops, especially the major fear is of `out crossing', or the flow of genes from GM plants to conventional crops, implementing indirect threats to food safety and security [12].

• GM mustard has mutated flowering and pollen production, as a result can affect honeybees directly or indirectly. Protease inhibitors have proved unfavorable to the longevity and behavior of bees.

  ❖ Advantage may out race the valuable Genetic Diversity
  ❖ Bt transgenes may adversely affects Monarch Butterfly

• Regulatory weakness–The Genetic Engineering Approval Committee, is authoritative for granting large-scale releases and commercialization of GMOs, works under the Ministry of Environment and Forests and is not a fully independent body [13].

Conclusion

In a current environment where climatic change would have negative effects on yield of many major crops which could seriously undermine food security, GM crops are the way forward in providing better yield to combat the shortage of food and also helps to eradicate the malnutrition in poverty ridden countries. However, to convince the bodies which are critics of GM crops to allow commercialization of GM crops we need a strong regulatory framework. We strongly needs an autonomous biotechnology regulatory authority, a single organization that will take over the multiple committees. This authority would consider the use of all GMOs in agriculture, pharmaceutical and biodiversity sector.

References

[8] https://entomology.ca.uky.edu/ef130