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BIOTECH BOUNTY ON VERGE: GM (GENETICALLY MODIFIED) CROPS AND THE SCIENCE OF SUSTAINABLE AGRICULTURE AND HORTICULTURE

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ABSTRACT

The global agricultural landscape is experiencing a transformative shift with the advent of Genetically Modified (GM) crops. This abstract delves into the profound impact of GM crops on the realms of sustainable agriculture and horticulture. Through the lens of biotechnology, this exploration highlights the scientific advancements that GM crops bring to the forefront, fostering a new era of agricultural sustainability. The discussion encompasses the innovative genetic modifications applied to crops, enabling resistance to pests, diseases, and adverse environmental conditions. These modifications not only enhance crop yield but also contribute to resource efficiency, minimizing the need for excessive pesticide and water usage. The abstract also examines the potential of GM crops to address food security challenges by increasing productivity and adaptability to diverse climatic conditions. Furthermore, the abstract explores the integration of GM crops in horticulture, showcasing their role in elevating the quality and nutritional content of fruits and vegetables. The precision and specificity offered by biotechnology in modifying plant genetics present opportunities to enhance desirable traits, such as taste, shelf life, and nutritional value. In addition to the scientific aspects, the abstract discusses the societal and ethical considerations surrounding the adoption of GM crops. It addresses concerns related to environmental impact, biodiversity, and the coexistence of GM and non-GM crops in agriculture. This exploration serves as a comprehensive overview of the biotech bounty that GM crops offer to the fields of sustainable agriculture and horticulture. By unraveling the intricate science behind these innovations, the abstract aims to contribute to a nuanced understanding of the potential benefits and challenges associated with the integration of GM crops into the agricultural landscape.

Key words : GM crops, Ethical, Biotechnology, Resistance, Vegetables, Fruits.

Introduction

Enhancing crop productivity and resilience to both living organisms and environmental factors can be readily accomplished by integrating traditional breeding methods with contemporary biotechnology approaches like "transgenics." Moreover, the production of transgenic crops provides a targeted and expedited method for enhancing crop quality (ACBIO, 2013). There are numerous procedures for transformation and regeneration, which have led to the development of a diverse range of horticulture crops with improved resistance to pests and

diseases, as well as longer shelf-life, among other benefits. The matters of public interest include the utilization of selectable marker genes, the dissemination of transgenes via pollen, the potential for the emergence of resistant strains in the event of insect or fungal pests, and the allergenic properties of the inserted proteins in humans. Presently, there exist methods to eradicate the utilization of selected markers in genetic transformation (ACBIO, 2013). Recently, numerous research groups and reputable organizations have evaluated the current status of the safety of genetically modified (GM) plants for human consumption. Their findings consistently indicate that transgenic crop varieties are equally safe and nutritious compared to their non-GM counterparts. The research and commercialization of transgenics face substantial challenges due to regulatory constraints (AFCD, 2017). Efforts should be expedited to accelerate the completion of essential toxicological research and issuance of required permission for the transgenic crop varieties. The utilization of advanced genome editing tools, like as ZFN, TALEN, and CRISPR/Cas9, has expanded the possibilities for creating genetically modified isogenic lines. These lines are designed to be both beneficial to consumers and environmentally sustainable. Moreover, they do not require much regulation due to their status as near-isogenic lines of the parental types (Ali *et al*., 2015).

Vegetables are cultivated on a global scale and have a significant impact on human nutrition due to their provision of vitamins, minerals, dietary fiber, and phytochemicals. Vegetables are linked to the enhancement of gastrointestinal health, promotion of healthy vision, and decreased susceptibility to heart disease, stroke, chronic ailments including diabetes, and certain types of cancer. The consumption and caloric contribution of vegetables to the diet exhibit significant variation based on geographical region, nationality, local customs and cuisine (Alonso-Prados *et al*., 1997). Vegetable production is adversely affected by many biotic stressors produced by pathogens, pests, and weeds, necessitating the application of substantial quantities of plant protection agents per hectare. US vegetable producers are reaping the advantages of cultivating genetically modified squash varieties that are immune to Zucchini yellow mosaic virus, Watermelon mosaic virus, and Cucumber mosaic virus. These modified squash cultivars were approved and made available for commercial use starting in the mid-1990s. Bt-sweet corn has demonstrated efficacy in managing some lepidopteran species and is widely embraced in the United States' fresh market (AP, 2016). Furthermore, new Bt-fresh-market hybrids are introduced annually. Similarly, genetically modified Bt-eggplant was selectively selected to minimize the need for pesticides and is anticipated to be cultivated by Asian farmers in the near future. Additional vegetable crops are currently being developed through genetic modification to augment their ability to resist insects and plant pathogens (including viruses), exhibit tolerance to herbicides and enhance characteristics such as delayed ripening to prolong the shelf-life of the produce, elevated nutritional content, seedless fruit and heightened sweetness (APHIS, 1992a). Transgenic plant breeding offers genetically modified seeds that incorporate advanced technology, which aids in integrated pest management in vegetable cultivation. This method reduces the need for pesticide sprays and enhances food safety by minimizing chemical residues. In addition, herbicide-tolerant transgenic crops can contribute to the reduction of ploughing in fields, resulting in fuel savings due to decreased tractor usage. This practice also safeguards the integrity of the soil by minimizing erosion ((APHIS, 1994a). Transgenic vegetable crops have the potential to significantly enhance sustainable vegetable output in the 21st century. Nevertheless, there are differences among countries regarding their levels of acceptability of transgenic crops. The success of biotechnology products hinges on the demonstration of distinct benefits and a high level of safety to both cultivators and consumers (APHIS, 2007a).

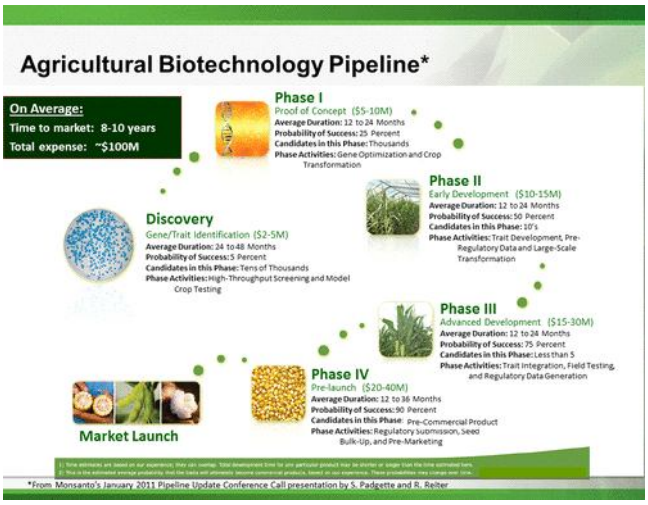

Fig. 1 : Biotechnology pipeline (source- google).

Historical context

The initial commercially available genetically modified (GM) product, known as the FlavrSavr™ tomato, was introduced to the US market in 1994. In the last two decades, the global land area used for cultivating genetically modified (GM) plants has reached a total of 185 million hectares. Nevertheless, genetically modified (GM) crops in horticulture make a relatively small and

frequently limited impact (APHIS, 2008).. The International Service for the Acquisition of Agri-biotech Applications (ISAAA) offers the most extensive reports on the integration of genetically modified (GM) crops into agriculture. These reports are backed by the US Department of Agriculture, the US Agency for International Development, various national and non-governmental organizations, as well as biotech companies (APHIS, 2009b).

GM events that have received approval are recorded in both national and international databases. These databases are overseen by several organizations such as the Biosafety Clearing House, the European GMO Initiative for a Unified Database System, the Center for Environmental Risk Assessment, and the FAO GM Foods Platform. Nevertheless, the accuracy of existing data on the cultivation of genetically modified (GM) crops may be compromised by variations in rules among countries and the absence of mandatory registration or labeling requirements in major producing nations. Private corporations sometimes restrict or withhold information about the growth of genetically modified (GM) crops from the public, and press communications are typically shared without independent evaluations (APHIS, 20014).

Officially sanctioned genetically modified plants

Tomato

The first genetically modified (GM) plant for human consumption was the FlavrSavrTM tomato, which was created by Calgene Inc. in 1994. Because tomatoes are fragile fruits, they are easy to bruise during harvest. The primary goals of tomato breeding were to increase fruit firmness or postpone softening, harvest at ripeness, decrease transport damage, and increase shelf life. It was suggested that genetic engineering methods, such RNA interference (RNAi) of the polygalacturonase (PG) gene, could facilitate the cultivation and dissemination of tomatoes (Aragao and Faria, 2009). Along with the Da, F and B tomatoes, Zeneca created the FlavrSavrTM tomato. The FlavrSavrTM tomato's gene constructs were introduced into the pCGN1548, pCGN1549, pCGN1158, pCGN1159 and pCGN1578 binary vectors. The events Da, F and B were created by transforming cotyledons taken from the T_7 tomato line using Agrobacterium. The transgenic plants were chosen after careful consideration of agronomic features evaluated in the field, the number of insertions, and the PG level. By employing polymerase chain reaction (PCR), the inbred lines and hybrids produced from them were molecularly confirmed to be homozygous for B, Da and F (Aragao *et al*., 2013). The results demonstrated that the full insert was present in the B and Da lines, however a deletion happened at the T-DNA right border region in the F line. The genetic alterations that were presented resulted in tomato fruits that were thicker and more consistent, had a slower rate of fruit softening, and had a 99% reduction in PG activity. The extended fruit hardiness was likely responsible for the transient delay in fungal infection. We did not detect any additional unintended impacts. In a Mendelian method, the transgene was stably implanted, passed down over generations and kept apart (Azadi *et al*., 2011). Although, the inserted PG gene did increase fruit firmness as expected, it had no effect on any other traits, and field testing comparing non-GM control plants to transgenic inbred and hybrid lines produced from the B, Da and F events revealed no significant agronomic changes. To find out where genetically modified crops stand in terms of regulation, Calgene started consulting with the FDA in 1989. In 1992, the FDA released new guidelines that stated genetically modified plant foods will be subject to the same regulations as conventionally grown foods (Bakum, 2015). To determine the regulatory status of FlavrSavrTM and to permit the presence of NPTII protein in tomato fruit, Calgene petitioned the FDA. The FlavrSavrTM tomato was deregulated by APHIS in 1992, and the FDA gave their approval in 1994. APHIS and the FDA deregulated Zeneca's Da, F and B tomato events after they went through similar processes. The 1401F, H282F, 11013F, and 70913F hybrids, which are produced from the transgenic F line, were also approved for food use by Health Canada. In 1995, the Advisory Committee on Novel Foods and Processes (ACNFP) in the United Kingdom gave its approval to a tomato paste made from the fruits of two F tomato hybrids (Basso *et al*., 2016).

In 1994, the FlavrSavrTM tomato made its debut on American shelves, accompanied by an easily readable label. The incorrect types used as parental material made it less firm than planned, which caused distribution problems and made it more expensive than a normal one. It was also not appreciated. This led to the 1997 recall of the FlavrSavrTM tomato. Rather than aiming for the fresh market, Zeneca selected their Da, B, and F tomato lines for processing. Commercially, the F line and its offspring were worth the most. Together, the Safeway and Sainsbury's grocery stores in the UK and Zeneca were able to sell 1.8 million cans of tomato paste made from tomatoes grown by Zeneca (BCH, 2012a). In 1999, when the backlash against foods made from genetically modified plants grew, Safeway and Sainsbury's chose to pull the products off the shelves, despite the voluntary labeling. In order to decrease fruit loss during distribution, other tomatoes that were approved underwent modifications.

The purpose of developing Events 35-1-N, 1345-4 and 8338 was to achieve a delayed ripening phenotype, which was believed to result in improved quality and longer shelf life. An extra nptII selection gene with nos promoter and ocs terminator was introduced with the 35-1-N event, which was developed by Agritope Inc. The event used a modified sam-k gene from bacteriophage T_3, which was controlled by a tomato fruit-specific E8 promoter and nos terminator (BCH, 2012b). One of Petoseed's cherry tomato varieties, Large Red Cherry tomato, was in the 35-1-N event's parental line. For the delivery of gene constructs, the A. tumefaciens EHA101 strain was utilized, which is vectorized using pAG 5402. To create the 1345-4 event, the tomato 91103-114 parental line was transformed with the pWTT2144/AccS vector using A. tumefaciens. The DNAs that were introduced were 1) a fragment of the ACS gene controlled by the CaMV 35S promoter, 2) nptII with the nos promoter and ocs terminator and 3) a fragment of the Cab22L gene leader. Reduced translation of natural ACC2 mRNA was seen in the presence of the truncated ACS. The company's patented technique, TranswitchTM, was instrumental in creating the 1345-4 tomato (BCH, 2012c). The 1345-4 tomato's genome contained three inverted T-DNA repeats, with one border deleted from each junction point (LB-LB, RBRB). Additional analysis revealed that the T-DNA was preserved in the offspring, with no signs of additional deletions or rearrangements. Monsanto created the 8338 event by transforming the UC82B tomato line with Agrobacterium. The transferred T-DNA segment included the ACCd gene from the Pseudomonas chlororaphis 6G5 strain, which was facilitated by a modified Figwort mosaic virus 35S promoter. It also included the HSP70 gene leader from Petunia × hybrida and the rbc-E9 non-translated region, which served as a polyadenylation signal (BCH, 2012d). The gene construct was shown to have been introduced into the plant genome in a single copy with a single ACCd and nptII gene, according to Southern blot analysis. With the exception of the desired alteration in delayed ripening, agronomic traits of the transgenic line and control plants tested in American fields in 1992 and 1994 were indistinguishable from one another. However, two GM hybrids exhibited an elevated redripe fruit percentage; this could be due to variations in the 'earliness' and 'lateness' of the other parent in the hybrids or variations in the penetrance of the delayed-ripening phenotype between backgrounds (BCH, 2012e).

In 1995, the FDA and APHIS deregulated Monsanto's 8338 tomato and the next year, they deregulated the 35-1-N tomato as well. 'Endless Summer' was the name given to the 1345-4 line for a brief period of time when it was authorized for production, feed, and human consumption in the UK in 1995. By overexpressing the Cry1Ac protein, a bioinsecticide utilized to shield conventional cultivars from pests, Monsanto created an insect-resistant tomato event, 5345. The insertion of the Cry1Ac gene was found to be an effective method of protecting tomatoes from many lepidopteran pests, according to laboratory investigations. These pests include tomato fruit worm, pinworm, hornworm, potato tuber moth, and cabbage looper (BCH, 2013a). The expression of the Cry1Ac protein protected tomatoes from pests more effectively and with less effort than foliar application of the insecticide. The 5345 tomato event was achieved by transforming the UC82B cultivar using *A. tumefaciens*. The PVLEBK04 border vector, which only had one gene, carried the cry1Ac gene from *Bacillus thuringiensis* subsp. kurstaki. It also had a polyadenylation signal provided by the CaMV 35S promoter and the 7S 32 untranslated region. This T-DNA vector also contained the aminoglycoside adenyl transferase (aad) gene, which has its own promoter and terminator and the nptII gene, which has the CaMV 35S promoter and nos terminator (BCH, 2013b). Stable integration of the gene construct was confirmed through seven generations after a single insertion was discovered in the transgenic event. Western blotting and enzyme-linked immunosorbent assays (ELISAs) were used to assess the AAD protein's expression, whereas Cry1Ac and NPTII protein levels were measured (BCH, 2014a). The Cry1Ac protein was found to be most abundant in immature leaves and least in fully ripe, red fruits. No alterations in growth or development were found in the 5345 GM line during the field trials that were done in the United States and Puerto Rico. In 1997, the business petitioned the USDA's APHIS and the FDA for approval of the 5345 event after conducting a safety evaluation. The transgenic line, however, has not been commercialized and has not been registered as a pesticide with the EPA (BCH, 2014b).

Fig. 2 : Genetically modified tomatoes.

Brinjal

In India, Bangladesh and the Philippines, especially among the poorer farmers, the eggplant fruit—also called brinjal or aubergine—is an essential food source and source of revenue. With the help of Cornell University and Monsanto, Maharastra Hybrid Seeds Company (Mahyco) created the first biotech food crop in India, the Elite event 1 eggplant. The eggplant fruit and shoot borer (FSB) was unable to damage this particular occurrence, which is responsible for yearly yield losses ranging from 51% to 73%. Due to the ineffectiveness of traditional breeding methods for pest management, harmful pesticides are used excessively, which has an adverse effect on the environment, the health of farmers, and the health of customers (BCH, 2014c). The EE1 event was generated by transforming cotyledons with a construct that included the cry1Ac gene from the *Bacillus thuringiensis* subsp. kurstaki HD73 strain, the CaMV 35S promoter and 7 S alpha terminator from soybean, the nptII gene controlled by the CaMV promoter and nos terminator, and the aad gene, which was not expressed in the plant due to its control by the bacterial Tn7 promoter. The transformation was mediated by *A. tumefaciens*. The plants were grown again and tested using a Southern blot assay in the subsequent generation (Bedrook *et al*., 1997). The results confirmed that the offspring exhibited the same pattern of limited pieces as the initial GM plant. Institutions in Bangladesh and the Philippines, as well as the Indian Institute of Vegetable Research (IIVR), Tamil Nadu Agricultural University (TNAU), and the University of Agricultural Sciences (UAS) all received free copies of the created technology. Bioassays conducted on EE1 hybrids between 2001 and 2009 revealed that they were extremely resistant to FSB, with insect mortality rates of 98% in shoots and 100% in fruit, compared to less than 30% in non-GM plants (Behboodian *et al*., 2012). In the Philippines, the University of the Philippines Los Banos was granted permission to conduct Bt eggplant field trials by the Bureau of Plant Industries (BPI). However, following a petition from the Masipag farmers' group and Greenpeace, the trials were halted by the Court of Appeals (CA). Now that the ruling has been affirmed by the Philippines' Supreme Court in 2015, Bt eggplant can move closer to commercialization. The Bangladesh Agricultural Research Institute (BARI) authorized the commercialization of four Bt eggplant varieties in 2013. From a low of 20 farmers in 2014 to a high of over 5,000 in 2016, the production of Bt eggplant began. Two thousand farmers across sixty-four districts will receive free genetically modified (GM) seeds and fertilizer from the government, which continues to support Bt varieties. Upcoming releases of three more Bt eggplant cultivars are in the works at the Bangladesh Agricultural Research Institute (Biology Discussion, 2016).

Fig. 3 : Genetically modified brinjals.

Squash

To combat potyviruses like Watermelon mosaic virus-2 (WMV2) and Zucchini yellow mosaic virus (ZYMV), Asgrow created two GM squash events, CZW-3 and ZW-20, that were resistant to these viruses. Another cucumovirus that the CZW3 event evaded was the Cucumber mosaic virus (CMV). Aphids are vectors for RNA viruses that produce stunted development, discolored fruit, and smaller leaves. The ZW-20 and CZW-3 events were derived from the cultivar 'Yellow Crookneck,' which was acquired through the transformation of leaf discs by Agrobacterium (Bommineni *et al*., 2000). Aside from the nptII gene, the T-DNA area included coat protein (CP) genes from the ZYMV FL and WMV2 NY strains. Fusing the 52 untranslated portion of the CMV cp gene with the ZYMV cp gene and the N-terminal sections of the WMV2 cp gene improved translation. In field testing conducted across the US, the CZW-3 and ZW20 transgenic lines were assessed. ZW20 plants were discovered to be resistant to both ZYMV and CMV, but CZW-3 plants exhibited no symptoms of CMV, WMV-2 or ZYMV (Bonfim *et al*., 2007). In comparison to the non-GM control plants, no other modifications were noticed on the GM plants. In 1992, Asgrow began working with the FDA to determine ZW20's non-regulated status; in 1994, the petition was accepted. The company no longer needed premarket evaluations or FDA approval after submitting a summary of a nutritional and safety assessment to the FDA in 1995. The following year, they gained an opinion that ZW-20 was not significantly different from non-GM squash varieties. Freedom II, a hybrid strain developed

from ZW20, first appeared on store shelves in 1995 (Brady *et al*., 1982).

Fig. 4 : GM squash.

Melon

Cantaloupe melon ripens quickly, leading to postharvest losses from overripe fruit and a diminished shelf life. Two GM events—A and B—with delayed fruit ripening were created by Agritope Inc. for melon. These events were created by transforming a construct with the sam-k gene under the control of the E8/E4 hybrid promoter and nos terminator, and the nptII gene with the raspberry RE4 promoter and gene 7 terminator from *A. tumefaciens*. The transformation was carried out by Agrobacterium (Bruening and Lyons, 2000). Through the use of polymerase chain reaction (PCR), Agritope was able to prove that the plant genome was unaltered and that only the T-DNA region was transposed into melon. There was a transient expression of the sam-k gene in ripe fruit but no SAMase in immature fruit or any other portion of the plant. The United States was the site of field trials involving both genetically modified (GM) lines and hybrids with non-GM types carried out by Agritope and Harris Moran Seed Company. The results demonstrated that the hybrids and lines both produced less ethylene, but the ripening time was only three days longer than the control (Cambra *et al*., 2006). The 1-3 day ripening delay was connected with the transgenic fruit's substantially greater quantities of soluble sugars compared to the control fruit. In 1998 and 1999, Agritope petitioned APHIS and the FDA, but only in 1999 were both lines approved for eating in the USA. Genetically modified (GM) melon is legal to eat, but its cultivation is not permitted due to the reversal of the deregulation process (Cervera *et al*., 2000).

Fig. 5 : GM melon.

Beans

A common bean resistant to the Bean golden mosaic virus (BGMV) was created by the Brazilian Agricultural Research Corporation (Embrapa) through the GM event Embrapa 5.1. The virus causes stunted development, pod deformity, and mosaic depigmentation of leaves. Tobacco whiteflies spread the virus, which reduces crop yields significantly throughout Latin America. Because currently available cultivars are susceptible to the BGMV, new strategies for resistance improvement had to be devised (CFIA, 2015). Following particle bombardment of the embryonic axis of the 'Olathe Pinto' embryo with the pBGMVRNAiAHAS vector, the Embrapa 5.1 event was produced. In order to post-transcriptionally silence the AC1 gene, the business opted to use RNAi technology, which involves inserting a double-stranded RNA hairpin structure. A sense and antisense oriented pair of AC1 genes, together with a CaMV 35S promoter and an ocs terminator, were part of the construct, which was homologous to a BGMV rep gene fragment (Chakrabarty *et al*., 2002). In addition, there is the als gene found in *Arabidopsis thaliana*. It encodes acetolactate synthase, a gene that confers resistance to imidazolinone and sulfonylurea herbicides. The als gene also has its own promoter and terminator. Out of eighteen transformants, the 5.1 GM line was selected for additional trials due to its resistance to the BGMV following plant infestation with virus-carrying whiteflies. After being exposed to more than 300 whiteflies per plant during its life cycle, around 93% of plants showed no symptoms. All non-GM bean plants, on the other hand, displayed signs of virus infection in response to as few as 2–10 whiteflies in a field setting (Chandrasekaran *et al*., 2016).

Molecular studies showed that the gene construct was introduced into a specific location in the plant genome and stayed put for multiple generations, even when crossed with commercial cultivars that were not transgenic. One full copy and three partial copies of the construct were found in the bean genome, according to subsequent investigations. It was determined that the genetically modified Embrapa 5.1 line of common beans was not herbicide tolerant, changed in any way in terms of phenotype or composition, and had no negative effect

on the environment when contrasted with the non-GM variety (Chen and Yang, 1996).

Papaya

The genetically modified fruit tree species papaya (*Carica papaya* L.) was first used for commercial production in 1996 and is being grown and sold today. Four genetically modified papaya events have been approved and created by scientists at universities in the United States or China. 'Sunset' cultivar 55-1 (OECD UID: CUH-CP551-8) and 63-1 (OECD UID: CUH-CP631-7), produced at the University of Hawaii and Cornell University, USA, were the first deregulated genetically modified papaya events (Choudhary and Gaur, 2009). 'Huanong No. 1' was unveiled by South China Agricultural University; it was not officially recognized by the OECD. In 2016, the X17-2 event (OECD UID: UFL-X17CP-9) created at the University of Florida was registered by the EPA and deregulated in the USA. Visible rings on fruits, mosaic, malformed, and smaller leaves are symptoms of the PRSV potyvirus, which is spread by aphids. Tree life expectancy is decreased from 20 to typically a few years, yields are dropped, and immature infected trees never bear fruit. Tree growth is also inhibited. Additional vectors for PRSV transmission include mechanical injury during pruning and seed transmission. Even while seed transmission is very small, it could help the pathogen spread to other areas. Complete orchard removal does not help manage the PRSV, and there are no appropriate control techniques. Although they still display indications of disease, several papaya cultivars that are currently available are somewhat resilient to certain types of viruses (COFEPRIS, 2018).

The PRSV impacts nearly every place that grows papaya, which is in the tropics. Papaya genetic transformation (GM) events 55-1 and 63-1 were developed as a result of research on virus coat protein (CP) that began in the late 1980s at the University of Hawaii in the USA. After being injected with a Hawaiian PRSV strain, transgenic plants were propagated. The gene construct included a chimeric PRSV CP gene, nptII, and the CaMV 35S promoter and terminator. The HA 5-1 strain was used to generate the PRSV gene, and the CaMV 35S promoter and nos terminator were used to control the uidA gene and nptII, respectively. The X17-2 event was developed through the use of the pBI121fs plasmid, a variant of the pBI121, which was mediated by *A. tumefaciens* (CTNBio, 2011). The PRSV cp gene from the H1K strain in Florida, together with the CaMV 35S promoter and nos terminator, and the nptII gene, also with the nos promoter and terminator, were all components of the plasmid's T-DNA. While the CP level was too low to be detected by ELISA, sequencing the insert in fifth-generation plants showed that the thymidine mutation in the cp gene had been rectified, allowing for translation of the protein detectable by Western blotting. To conduct a resistance test in a controlled environment, the Hawaiian virulent PRSV HA strain was introduced to 55-1 and 63-1 plants. For the full six months following inoculation, only the 55-1 plants showed no signs of illness. Subsequent testing confirmed that 55-1 plants could withstand other strains indigenous to Hawaii, but not those from anywhere else in the globe. From 1999 to 2007, five X17-2 offspring pollinated with non-GM types were tested for resistance to the three PRSV strains found in Florida: H1K, H1C and H1A. Hybrids produced from the PRSV-resistant X172 variety show promise for Florida papaya growers. Cornell and the University of Hawaii petitioned the USDA's APHIS in 1996, arguing that the 55-1 and 63-1 events should not be controlled since they did not pose a threat to plant pests. Following 2008 consultations, the FDA determined that the 55-1 event did not pose any significant differences in composition, safety, or other important criteria compared to existing papaya varieties. Consequently, the FDA did not deem it necessary to conduct premarket evaluation or approval. The FDA did not consult on the 63-1 incident (Dahmani-Mardas *et al.*, 2010). The FDA ended similar consultations on the X17-2 event in 2008, and by 2009, the event has been deregulated in the US. In 2016, the X17-2 papaya was officially registered as a Plant Incorporated Protectant (PIP) with the EPA, in compliance with FIFRA section 3(c) (5). After reviewing the 55-1 event, Health Canada determined that the 'Rainbow' and 'SunUp' papaya varieties were "as safe and nutritious as currently available commercial papaya varieties" and did not pose any risks to human food safety. In 2011, Japan also gave its stamp of approval to the 55-1 event for import and culinary use. After realizing that GM papayas accounted for more than half of the market and that GM trees were widely planted, Hong Kong finally gave in and removed the ban on genetically modified papayas in 2012 (Dale *et al.*, 2017). A 'SunUp' cultivar that is homozygous for PRSV resistance and produces red-fleshed fruits was developed from resistant 55-1 plants. In the field, GM varieties 'SunUp' and 'Rainbow' both grew PRSV-resistant fruits of excellent quality. Crossing GM and non-GM papaya eventually yielded other types that weren't as important for world production.

In 1998, the individuals or organizations responsible for the development of genetically modified papaya (GM papaya) granted licenses to the Papaya Administrative

Committee, an organization that represents papaya growers. Farmers in Hawaii were given free 'SunUp' and 'Rainbow' seeds in 1999, the same year that the first harvest took place. By the turn of the following decade, 'Rainbow' trees had spread throughout forty percent of the papaya-growing area. After falling from 26,000 metric tons in 1992—the year the PRSV outbreak occurred—to 16,000 metric tons in 1998—the adoption of genetically modified (GM) types immune to the virus saved Hawaii's papaya crop. 'Rainbow' trees, which produced 25 times more fruit than the non-GM 'Sunrise,' were largely responsible for the rapid recovery of papaya output after the 1998 release of genetically modified (GM) seed (Dellanay *et al*., 1989). By 2001, production had increased to 24,000 metric tons. In 2015, about 4,000 tons of papaya were exported from the United States; the majority (74%), shipped to Canada, followed by Japan (11%), Hong Kong (5%), and other nations. There have been multiple efforts in recent years to restrict or ban genetically modified plants in Hawaiian counties, leaving farmers worried about their future production. The United States Court of Appeals for the Ninth Circuit reversed a draconian anti-GMO law that had been passed in 2013 in 2016.

Fig. 6 : GM papaya.

Plum

The 'HoneySweet' genetically modified plum variety, created by the USDA-ARS Appalachian Fruit Research Station, is able to withstand the infection caused by the Plum pox virus (PPV), or Sharka. When it comes to stone fruit orchards, sharka is by far the most serious disease. Fruits from diseased trees are often misshapen and fall off trees before they reach full ripeness, rendering them useless for human consumption or industrial use. Worldwide, the cost of managing PPV infection has surpassed 10,000 million euros in the last 30 years, and in Europe alone, annual yield losses owing to infection are estimated at 1.5 million tons. Eradication initiatives are the primary means of control, and they have temporarily limited the spread of PPV in some nations including the United States (Embrapa, 2016). "HoneySweet" was created by transforming hypocotyl slices in a controlled laboratory setting using *A. tumefaciens*. Following inoculation with PPV strains D and M, which were transferred by aphids, the transgenic shoot (No. C5) was able to root and pass the initial resistance tests conducted in a greenhouse. The PPV coat protein is rendered inactive and absent from any tree cell in 'HoneySweet' due to the fact that its resistance mechanism is dependent on post-transcriptional gene silencing. Genetic engineering resulted in faulty multicopy insertion of the gene construct into plum genome, as shown by restriction analysis and sequencing (EPA, 2010). There are five known genetic structures, one of which requires an inverted PPV-CP sequence repeat to be present. Short interfering RNAs, a byproduct of degrading this RNA species, are essential for further degrading the entire target RNA molecules. As a result, the PPVCP RNA that is transgene-transcribed in 'HoneySweet' is degraded, guaranteeing that the constitutive resistance response is maintained even in the face of PPV infection, as well as the transcription of newly imported viral RNA. Tree resistance to the PPV has been established through six- to ten-year field tests of 'HoneySweet' trees in the Czech Republic, Poland, Romania, and Spain, where the PPV is endemic. Even after being inoculated with PPV-infected grafts, 'HoneySweet' exhibited relatively minor symptoms, despite the fact that it was naturally infected by the aphid-transmitted virus. Sour Cherry, El Amar, D, and M are all PPV strains that have proven resistant (EPA, 2014). As per FIFRA section 3(c)(5), the EPA designated 'HoneySweet' as a new PIP (Plant Incorporated Protectant) for a duration of one year in 2010. Growing and eating 'HoneySweet' in the United States will not be affected by the ruling. Sharka remains a major concern because the PPV causes extensive damage and there is currently no effective chemical control to prevent or eradicate the virus. The only way to manage the spread of the disease is to cut down afflicted trees.

Fig. 7 : Genetically engineered plum.

Pineapple

The Del Monte Fresh Produce firm, which is based in the United States, is the firm that developed genetically

modified pineapple (GM). The 'Extra sweet pink flesh pineapple' (EF2-114 event) is the only genetically modified variety that has been approved up to this point. It is a modified MD2 variety that accounts for ninety percent of all exports around the world. Due to the accumulation of carotenoid and the management of flowering, the genetically modified pineapple is designed to have alterations that result in the fruit flesh being red or pink in color. There is a health-promoting red lycopene that accumulates there at a mean level of 21 ppm, which is comparable to the level that is found in other red-colored fruits such as tomato, watermelon, grapefruit, and papaya (EPA, 2016). This is the primary factor that contributes to the unique color of the flesh. The newly introduced red color is not only appealing to customers, but it also makes it simple to distinguish the genetically modified pineapple from the conventional yellow pineapple. The genetically modified pineapple was created by the process of transformation mediated by *A. tumefaciens*. Separate T-DNA gene constructs were created and injected into the GV3101 (pMP90) *A. tumefaciens* strain. These constructions were inserted into the strain. The initial plasmid, known as pHCW.T-7, was composed of four cassettes: 1) the phytoene synthase (psy) gene from tangerine (*Citrus unshiu*), 2) and 3) a partial coding fragment of the β-lycopene cyclase (Lcyb) and ε-lycopene cyclase (Lcye) genes in the sense and anti-sense orientation, and 4) a mutant acetolactate synthase (ALS) gene from tobacco (SuRBHra), conferring chlorsulfuron resistance due to the replacement of two amino acids in the wild type ALS molecule. A partial coding fragment of the pineapple meristem ACS gene (flACC3') was present in the second plasmid, which was designated as pHCWflACC3'-2. This fragment was oriented in both the sense and anti-sense directions (Ferreira *et al*., 2002).

The selection of chlorsulfuron-tolerant clones and their subsequent cultivation for the first time occurred in 2008, following a series of transfers and micropropagation. Over the course of the following year, plants were grown in a greenhouse in Costa Rica for fifteen to twenty weeks, after which they were moved into the soil for field experiments. The clone that performed the best, the EF2-114 event, had a morphological appearance that was comparable to that of the MD2 variety. Further, it had a high lycopene content and lower levels of â-carotene. The genetic insertion was stable over the course of four generations of vegetative development, as demonstrated by the findings of molecular investigations, which revealed up to four copies of the injected vector pieces. The developer had the intention of cultivating genetically modified pineapple in Costa Rica and in 2011, its subsidiary, LM Veintiuno, was granted permission by the National Technical Commission on Biosafety of the Ministry of Livestock and Agriculture to plant an area of up to 200 hectares. For the year 2016, the total area of production from those fields in Costa Rica, which are only permitted for export, reached 226 hectares. The Food and Drug Administration (FDA) conducted an investigation into the regulatory and safety concerns that were associated with human food that was generated from the EF2-114 pineapple type. The FDA came to the conclusion that there were no unresolved safety or regulatory concerns surrounding the genetically modified pink flesh pineapple that were governed by the Federal Food, Drug, and Cosmetic Act (FFP, 2017).

Fig. 8 : Pink GM variety of pineapple.

Apple

In recent years, the emergence of genetically modified apple types has become a reality. Some examples of these varieties include Arctic Golden, Arctic Granny, and Arctic Fuji. Okanagan Specialty Fruits Inc., which was purchased by American Intrexon Corporation in 2015, is responsible for the development of three Arctic® apple types that are now permitted for production and consumption with proper authorization. As a result of the slowed enzymatic process of oxidative browning that often takes place in apple fruits that have been wounded, these types generate fruits that do not become brown, when they are chopped, sliced, bitten, or bruised (Fichtner *et al*., 2014). The activity of polyphenol oxidases (PPOs) is responsible for the process. These enzymes involve the conversion of phenolic compounds to quinones in the presence of oxygen. The quinones that are produced as a result of this conversion polymerize to create brown melanins. In addition to being a significant contributor to postharvest and processing fruit loss, browning also has a negative impact on the shelf life of apples, which consumers of fresh and sliced apples do not appreciate. Due to the fact that four PPO genes were silenced

through the use of RNA interference, Arctic apples have a demanding non-browning phenotype. A. tumefaciens was used to facilitate the transformation of leaf segments that were removed from plantlets that were grown in vitro of common varieties such as "Golden Delicious," "Granny Smith," and "Fuji." This allowed for the development of these techniques. The EHA105 strain of A. tumefaciens included a binary GEN-03 vector that had been disarmed (Firoozbady and Young, 2015).. This vector was a derivation of the plasmids pBINPLUS and pBIN19. Both the first cassette, which contained the nptII gene and was controlled by the nos promoter and the nos terminator, and the second cassette, which was composed of the duplicated-enhancer CaMV 35S promoter and the nos terminator, flanked the 1.81 kb chimeric PGAS insert that was constructed of four 394-457 bp long apple PPO gene fragments in the sense orientation, were contained within the DNA fragment of GEN-03.

The results of the Southern analysis showed that the 'Arctic Golden' strain had two unlinked T-DNA insertions, whilst the 'Arctic Granny' strain had four unlinked copies. According to the findings of the entire apple genome sequencing carried out with Illumina technology, the 'Arctic Fuji' variety featured numerous insertions in three of its chromosomes. The insertion of anptIIgene into each of the three kinds resulted in the expression of a single new functional protein known as NPTII. This protein is significant for the selection of transgenic events during the development process. Fruits of the Arctic® types did not differ significantly from those of donor varieties in terms of the amount of moisture, calories, sugar profile, protein, carbs, dietary fiber, and potassium that they contained. A silencing strategy that makes use of RNA interference was utilized in order to generate the Arctic apple variety, which was derived from the PPO2, GPO3, APO5 and pSR7 genes (GAIN, 2011). In order to decrease the expression of the complete apple PPO gene family in a transgenic plant, the PGAS transcript was utilized. In order to facilitate shoot regeneration and micropropagation, leaf segments were subjected to selection media after the transformation process was completed. Plantlets that had a PPO activity that was reduced by more than 80 percent were chosen for further molecular analysis and characterization. There were two unlinked T-DNA insertions found in the Arctic varieties, while the 'Arctic Granny' variety had four unlinked copies. Based on the results of entire apple genome sequencing performed using Illumina technology, it was discovered that the 'Arctic Fuji' apple featured numerous insertions in three of its chromosomes (Gonsalves, 2004). The insertion of anptII gene into each of the three kinds resulted in the expression of a single new functional protein known as NPTII. This protein is significant for the selection of transgenic events during the development process. Fruits of the Arctic types did not differ significantly from those of donor varieties in terms of the amount of moisture, calories, sugar profile, protein, carbs, dietary fiber, and potassium that they contained. APHIS of the United States Department of Agriculture came to the conclusion that GD743 and GS784 plants are not expected to be a threat to plant pests and would not have a substantial influence on the quality of the human environment or endangered species. This led to the beginning of the deregulation process in the United States in the year 2012. Following the conclusion of consultations in 2015, the Food and Drug Administration (FDA) stated that events GD743 and GS784, as well as the meals and feeds generated from them, do not differ from comparable apple types that are already cultivated, marketed, and consumed in the United States in terms of composition, safety, or any other relevant attribute. On a total of 80 hectares of land in the United States, Okanagan Specialty Fruits planted 70,000 'Arctic Golden' and 'Arctic Granny' trees in 2016. Additionally, the company contracted for an additional 800,000 plants to be planted over the course of the subsequent two years. 'Arctic Gala', the fourth kind of Arctic apple that does not brown, will be introduced in the near future, according to the firm, which has also indicated that the first harvest from commercial production was announced in 2017 (Gonsalves *et al*., 2004).

Fig. 9 : GM arctic apple.

Corn

Corn is the only crop that is commercially grown and sold in five European countries. It is responsible for the production of roughly 173 million tons of ensilage maize and 56 million tons of grain maize. A portion of the Bt corn seeds are utilized in the production of several food items, including corn oil, corn oil, corn on the cob, corn flakes, popcorn, and canned sweet corn. It is mandatory for all food products that are made from Bt corn to be labeled in Europe due to regulations. The United States of America and Canada, on the other hand, do not have such regulations and about seventy-five percent of their produced maize products are made from Bt corn. 1997 saw the beginning of the cultivation of Bt maize in the

United States of America, Canada, and Europe (Spain), and by 2009, it had been planted commercially in eleven different nations. It was estimated that there were 60.6 million hectares of genetically modified maize in the world by the year 2016 (HC, 1995a). Of this total, 6 million hectares (10%) were Bt corn, 7 million hectares (11.7%) were herbicide-tolerant corn, and 47.7 million hectares (78.7%) were combined Bt and herbicide-tolerant corn. In the beginning, the crop was developed to protect against the infestation caused by the European corn borer, also known as *Ostrinia nubilalis*. However, as the 2000s progressed, it was also developed to protect against the corn earworm, also known as H. zea, and the corn rootworm, also known as *Diabrotica virgifera*, in addition to those two pests. A number of countries around the world, including Brazil, Argentina, India, Canada, China, and South Africa, have been quick to adopt genetically modified (GM) features. The United States of America is one of these countries. GMOs were used to cultivate 93 percent of all soybeans and 88 percent of all corn in the United States in the year 2012. There has been a steady increase in the number of corn hybrids in the United States that have more than one genetically modified characteristic, reaching 52% in 2012 (HC, 2015). Transgene flow include not just the gene of interest but also additional genetic elements that are present in the transgenic construct. These elements include promoter, terminator, and marker genes, in addition to related non-transgenic genes of the host genome that "hitchhike" along with the transgenes. The United States of America is the world leader in the development, promotion, production, and regulation of genetically modified animals (GMO) crops. In 2005, 49.8 million hectares (ha) of GMO crop area were planted in the United States. The majority of 328 farm households in Mexico, Cuba, and Guatemala were in favor of genetic engineering (GE), but an even larger majority (86%) were not willing to accept the potential future consequences of a hypothetical GE variety. These potential consequences include reliance on the formal seed system and yields that were initially high but have since decreased due to pest resistance. Changes in farming practices, economic and social shifts, as well as national and international policies that undercut the viability of conventional agricultural systems, could all have an impact on the diversity of maize (Kato *et al*., 2010).

There is a possibility that the current threat to maize diversity in Mexico is not exclusively caused by the introduction of transgenes into conventional agricultural systems; rather, it may also be the result of national and international policies that impair the viability of those cultivation methods. The notion that small-scale maize cultivation in Mexico should and would disappear was one of the economic assumptions that served as the foundation for the North American Free Trade Agreement (NAFTA). Over the course of just three years, tariff protection for Mexican maize producers was gradually eliminated (Lisa and Lecoq, 1984). This led to an increase in the amount of transgenic maize that was imported into Mexico as grain and a decrease in the sustainability of traditional agricultural systems in Mexico. There is a high probability that transgenes are present in Mexican maize FVs and that they may have introgressed into them; nonetheless, the evidence that is currently available is insufficient to draw definitive conclusions. There is no indication that the existence of transgenes in Mexico has directly harmed maize biodiversity, despite the fact that it is plausible that genetically engineered (GE) varieties could have a number of different types of direct effects on maize diversity. But it is difficult to estimate the extent of these effects. There are potential adverse consequences on diversity, and these effects could become even more severe if genetically engineered maize varieties, which are currently being developed for the production of medicinal and industrial chemicals, are commercialized (Luis-Arteaga *et al*., 1998).

Fig. 10 : BT corn.

Cotton

There was a time when Mexico's cotton fields used massive amounts of chemical pesticides. Midway through the twentieth century, Mexico's cotton fields expanded to 900,000 hectares, yielding 2 million bales of cotton, or "white gold." Years later, though, insect resistance to chemical pesticides evolved in response to mounting pest pressure and heavy pesticide dosages. In addition, unsustainable operational costs caused production to fall as a result of falling worldwide fiber prices. Because to the extensive use of pesticides in cotton production prior to the invention of Bt cotton, the industry incurred enormous monetary, environmental, and health-related expenditures. The farmers realized they needed to change their approach if they wanted higher yields, so they

started using genetically modified (GM) cotton cultivars that had genes added to them that made them resistant to herbicides and lepidopteran pests (MOEF, 2010). While conventional cotton yields were expected to be 5% higher, a research out of the University of California found that Bt cotton fields reduced pesticide costs by an average of $25 to $65 per acre from 1996 to 1998. Bollgard cotton, which produced the Cry1Ac toxin, had great action on pink bollworm and tobacco budworm, and was the first Bt cotton to be sold in the United States in 1996. The pink bollworm was the primary target of Bt cotton in the Western Cotton Belt, whereas tobacco budworm, autumn armyworm, *Spodoptera frugiperda* and *S. exigua* were less of an issue in the Mid-South and South-east regions of the United States. India and China saw a significant increase in Bt cotton farming in 2006 and 2007, reaching 25 million acres (2.5 million ha), making it the sole Bt crop farmed in developing nations (Polak *et al*., 2017). Of the 35 million hectares (ha) of cotton grown globally in 2016, 22.3 million (or 64% of the total) were genetically modified (GM) varieties. Of the 4 million hectares (ha) of cotton grown in the US, 3.2 million hectares (80%) were hybrids of Bt and herbicide-tolerant varieties. Eleven distinct Bt toxin combinations were produced by the 18 registered Bt corn and Bt cotton varieties in the United States. Caterpillars and beetles, or both, are killed by the 1-6 Bt toxins produced by each kind. The distribution of wild cotton species was analyzed using data from the CONABIO database, which contained information on sixteen different cotton species. In general, farmers noted that GM cotton increased yields while simultaneously improving pest control and making pest management easier. The increased usage of pesticides and the high cost of genetically modified cotton seeds were also pointed out (Scorza *et al*., 2016). Optimal growing circumstances and high-quality seeds typically account for the greatest GM cotton yields. Northern Mexico is desert and subject to extreme weather, which drives up the expense of producing cotton. Variations in global fiber pricing and high operational costs caused the total cotton area grown to fluctuate greatly. Due to their effective control of lepidopteran pests and outstanding weed management, genetically modified (GM) cotton types have 80% of farmers highly satisfied, despite the high production cost. Eleven percent are only somewhat content, and nine percent are completely dissatisfied. Only 10% of farmers think genetically modified (GM) cotton is a money loser. Forty percent of Mexican farmers would use conventional seeds if they were accessible because they think they would be cheaper. Moreover, they hold the view that genetically modified (GM) crops are not always necessary for controlling the pest populations that have been noticed in recent years. Farmers view the use of genetically modified (GM) cotton in a positive light, particularly with regard to human health. They think that the use of genetically modified (GM) cotton has decreased the number of cases of intoxication caused by exposure to chemical pesticides. They reduced the usage of chemical pesticides, which led to fewer reports of poisoning (Scorza *et al*., 2016).

Field seasons for annual crops, like cotton, last about six to seven months and necessitate heavy weed and insect pest management. Lepidoptera insects are the only ones that can be affected by the Cry toxins that are expressed in various Bt cotton events (Bt cotton). Toxins like these are effective against many cotton pests, including *P. gossypiella*, *H. zea*, *H. virescens* and *S. exigua*. Nevertheless, synthetic insecticides are still necessary for controlling the coleopteran *A. grandis*, the hemipteran *B. tabaci* and other insect pests that attack cotton. Because of its great specificity and capacity to eliminate certain pests, Bt is utilized in organic agriculture and integrated pest management (IPM). Despite the fact that Bt cotton reduces lepidopteran pests, other cotton pests that Bt cotton does not control may instead grow in number. This has been noticed all around the world, which means that secondary pests might take over resources that were once occupied by lepidopteran insects. Additionally, non-target organism populations may be more volatile in conventional cotton fields as opposed to Bt cotton fields. Beneficial insect populations may see an uptick if broad-spectrum pesticides are used less frequently. Having fewer lepidopteran eggs and larvae in Bt cotton, nevertheless, can change the food sources and hosts of natural enemies. In conclusion, there are differing views on the environmental impacts and consequences on non-target insect variety caused by the adoption of genetically modified (GM) cotton in Mexico (Siddique, 2017).

Fig. 11 : BT cotton.

Soyabean

In the battle against genetically modified (GM) allergies, soybeans—a food staple in Asia and a component in many processed foods—have taken center stage. The majority of genetically modified (GM) soybeans grown in the United States are resistant to herbicides. For many purposes, including food, animal feed, and industrial precursors, genetically modified soybeans hold enormous promise as a source of new and improved proteins and lipids. People who are health-conscious and/or have sensitivities to lactose or milk proteins are driving the rising popularity of soy milk and other dairy product substitutes. Atopic responses and stomach distress are the main adverse reactions to soybeans, and it is estimated that 5-8% of children and 1-2% of adults have them. Even though there is limited access to genetically modified (GM) soybeans in the UK, critics of biotechnology argue that the apparent increase in cases of soybean allergies in the country is linked to their development for the American market. Soybean products and processed foods have recently been widely available and accepted in the UK marketplace, which helps to explain the increases in soybean sensitivity. Animal meals made from soybeans are supplemented with other amino acids to make them more balanced because soybeans are low in methionine. Adding methionine to legume seeds and lysine to cereal seeds were the initial targets of transgenic plant studies aimed at enhancing seed amino composition for consumer characteristics (Svitashev *et al*., 2016). Tobacco and other model plant seeds were used in early research to demonstrate the transferability and expression of genes expressing seed proteins with increased methionine content. This allowed these foreign proteins to supplement the total amino acid content. It is possible to increase methinione content by transferring and expressing 2S albumins from tree nuts, such as Brazil nuts, which are abundant in methionine. Since soybean protein is present in a significant portion of processed and prepared foods in industrialized nations, it would be advantageous to produce soybeans that are hypoallergenic. Although avoiding meals containing soybean protein is the mainstay of treatment for food allergies at the moment, hypoallergenic versions of these foods may help lessen the likelihood of adverse reactions and make them more accessible to those who are sensitive. Creating an allergen-free soybean crop is possible through a combination of methods such as gene suppression, epitope modification, protein engineering, and allergen-free cultivars. By searching germplasm collections for cultivars that do not produce allergies, it is possible to breed elite germplasm with these varieties. The likelihood of a naturally occurring variant with enough changes to disrupt allergenicity is incredibly low, nevertheless, due to the abundance of different linear epitopes. Through the use of linear peptides, protein engineers can potentially modify amino acid sequences by removing allergenic regions (Tennant *et al*., 1994).

Studies on peanut allergies using the epitope modification approach have shown that it is possible to create a version that is essentially hypoallergenic. Nevertheless, putting this technique into practice is a challenge due to the need to entirely eliminate the intrinsic allergen and replace it with the 'hypoallergenic' version. Further, the protein's structure may change as a result of the allergenic epitope removal process, which in turn could impact the protein's capacity to accumulate, stabilize, and target within cells. Another way to use genetic modification (GM) is to inhibit the allergen so it disappears. Ragweed and rye pollen allergens, as well as a rice seed allergy that was reduced fivefold, are examples of plant allergens that have been reduced or eliminated by gene suppression technology. Also, the main allergen of domestic cats and a shrimp allergen that could be harmful to sensitive persons have both been the subject of experimental suppression. The immunodominant human allergen P34/Gly m Bd 30k was removed from transgenic soybeans created by researchers using gene-silencing techniques. Extensive proteomic research has shown that it is possible to inhibit an endogenous allergen in soybean seeds without harming the plants or altering their genetic makeup beyond removing the specific protein of interest. The GM soybean seed is nearly indistinguishable from the non-GM seed in every way, save for the one targeted trait that has been altered, thereby passing the "substantial equivalence" criteria (Yeh *et al*., 2011).

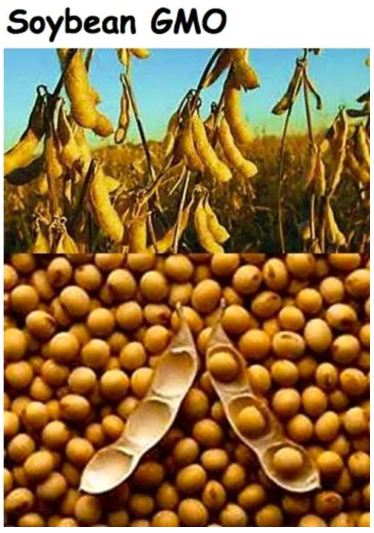

Fig. 12 : GM soyabean.

A first step in addressing the rising concerns about food allergies and their relationship to the development of GM crops is suppressing P34/Gly m Bd 30k in GM soybeans. Countries that are wary of this technology now should be able to get regulatory permission with the help of more thorough investigations and methods. Genetically modified (GM) crops do not appear to present any dangers that are comparable to, or even less severe than, those caused by naturally occurring plant allergies. There is an immediate need for improved biochemical and molecular techniques, such as animal models, to conduct experimental testing for food allergies in order to widely use genetic modification to crops (Yokotani *et al*., 2009).

Mustard

Increased food security, higher crop yields, and higher farmer profitability are all results of hybrid technology's impact on alleviating poverty. Two such examples are the 1940s American corn revolution and the 1980s Chinese rice revolution. In order to break yield limits, leading oilseed producers such as China, the United States, Canada and the European Union have switched from open-pollinated rapeseed varieties to hybrids. But as a result of slower increases in mustard and other crop yields in India, imports have been on the rise and are becoming unsustainable. Heterosis, the product of hybridization, can cause crop yields to skyrocket, outperforming both parent lines. Researchers at India's University of Delhi found that heterosis could be a key to unlocking hybrid vigor by crossing Indian and East European lines. Since mustard flowers have both male and female organs, they are mostly self-pollinating, which made it difficult to achieve hybridization through cross-pollination. The creation of male sterility as a pollination control mechanism with an inbuilt mechanism to restore fertility is a less burdensome and cost-effective approach for hybridization. The induction of male sterility, subsequent restoration of fertility, and maintenance of seed purity are the three pillars upon which a viable hybridization technology rests. While cytoplasmic male sterility (CMS) presents a potential solution for hybridization in self-pollinated crops, it is not always feasible to maintain male sterility in large-scale seed production using conventional plant breeding procedures. In some cases, genetic modification offers hope by making males permanently infertile regardless of environmental factors and then re-fertilizing them to create hybrids from pure seeds. The resultant male sterility systems that incorporate GM technology are highly adaptable and versatile.

Celestine Mariani and her colleagues at Belgium's Plant Genetic Systems (PGS) created the first genetically modified (GM) male sterility system for rapeseed. This system was quickly adopted worldwide and effectively integrated into rapeseed production in the US, Canada, and Australia. In 2002, using a modified version of the PGS's methodology, the University of Delhi created the first genetically modified hybrid, DMH-11. Field trials were carried out at ICAR-affiliated universities and research institutes to assess the yield benefit of genetically modified mustard DMH-11. The "Central Compliance Committee" was formed by the government to oversee these trials. The panel of specialists was selected by the DBT, the MoEFCC, and the ICAR.

Consistently greater yields compared to parents and local comparators were seen in the lengthy list of field studies that concluded with multilocation testing. Constrained field trials began in 2004, multilocation trials in 2006–2007, biosafety research level (BRL) I trials in 2010–2011 and 2011–2012, and BRL II trials in 2014–2015, all conducted at different sites, as part of the process of producing the transgenic DMH-11 hybrid. Results from the three BRL studies conducted in eight different locations demonstrated a 37% increase in yield compared to the national check variety when aggregated. In addition, the hybrid GM mustard planted with EH-2 modbs2.99 and Varuna bn3.6 produced greater yields than either parent line alone. This discovery proved beyond a reasonable doubt that the seed production system based on transgenic technology produced hybrid vigor, also known as heterosis. Achieving a mechanism for producing hybrid seeds efficiently is an exceptional achievement. It is possible to use the offspring's bn3.6 and modbs2.99 as a basis for a number of novel hybrids that combine more modern types with improved yield potential. The yields of future hybrids will be considerably greater than those of DMH-11, which is just the first generation. Similar to the success of Bt cotton, the next generation of transgenic hybrids has the potential to bring forth much more progress in mustard production and development. Methodical, organized, and multi-tiered is the regulatory framework in place for genetically modified crops in India. According to the "Rules for the Manufacture, Use, Import, Export and Storage of Hazardous Micro Organisms/Genetically Engineered Organisms or Cells, 1989" that were announced under the Environment (Protection) Act, 1986, these regulations have been prepared. This regulatory framework was put into place by various DBT and MoEFCC committees in relation to the GM mustard case. For more than 20 years, the genetically modified mustard was overseen by this complex regulatory framework. The technology was recommended for environmental release in October 2022 after the GEAC pronounced it

safe for food, feed, and the environment after extensive testing (GoI, 2022). The results of the experiments proved that no unexpected molecular changes had occurred, hence we can say that the plants were genetically identical with the exception of the targeted modifications. After the molecular characterization, the results of the compositional studies on the parental lines and DMH-11 were in agreement with the latter's conclusions. The seeds and leaves were found to be safe for consumption according to toxicity tests carried out at the FDTRC of the NIN. Additionally, weediness and aggressive growth patterns were not identified as threats to biodiversity in environmental studies. In the absence of natural selection, tests revealed that honeybee foraging levels were same, that soil microflora was present, that there was no transfer of genes between species, and that there was nearly no likelihood of pollination across species.

Fig. 13 : GM mustard.

Whether or whether genetically modified foods generate allergies

There is a lot of worry among consumers because, according to those who oppose genetically modified (GM) crops, new foods can cause allergic reactions. Leaders in southern African nations have rejected food aid from the United States in an effort to avert starvation, citing fears of genetically modified crops as the reason. This is despite the fact that the food being supplied is identical to the GM items that the majority of Americans safely consume. There have been thousands of genetic alterations made to plants by genetic engineering, which is now normal practice for scientific research. Experiments are typically carried out on model plants or agricultural plants such as rice, tomato, and tobacco with no intention of releasing or commercializing the results. Accurately predicting which proteins might be food allergens is challenging. The World Health Organization created a decision tree that includes questions regarding whether the protein shows traits that could make it more likely to be an allergy. This decision tree can only serve as a general reference; it cannot be relied upon as an accurate and reliable predictor of allergic potential or potency. There is a public database accessible online that contains allergenic proteins and epitopes. Different members of the same gene family can trigger an allergic reaction in quite different ways. Some members of the 2S family of seed storage proteins, for instance, do not seem to be allergies, although they contain some of the most powerful and harmful plant allergens.

The use of biotechnology to create genetically modified crops has sparked rising public awareness and anxiety about food allergies. People who are already sensitive to certain foods can develop food allergies, and their reactions can get worse with each subsequent encounter. About 2% of adults and 5-8% of children have actual food allergies; the rest typically have other dietary issues, like lactose intolerance. 'Hygiene hypothesis' is one of several explanations put out to account for the apparent rise in food allergy cases; it postulates that cleaner modern lifestyles cause fewer immunological challenges in early life, which in turn makes people more sensitive to subsequent immunological challenges, ultimately leading to food allergies.

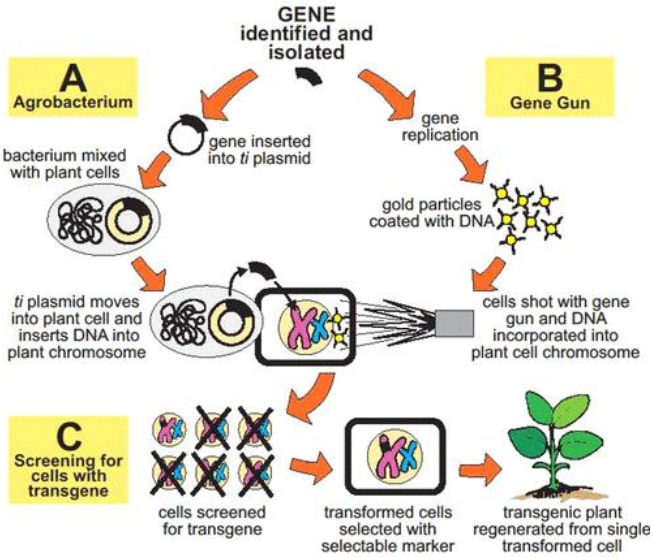

Fig. 14 : Process of genetic engineering.

Conclusion

In conclusion, the Biotech Bounty heralds a transformative phase in agriculture and horticulture with the advent of Genetically Modified (GM) Crops. The intersection of biotechnology and sustainable farming practices holds immense promise for addressing global challenges such as food security, environmental sustainability, and resilience against changing climates. The enhanced productivity and resource efficiency offered by GM crops can significantly benefit farmers and contribute to more sustainable agricultural systems. However, a nuanced approach is imperative, considering

the ethical implications, environmental concerns and public perceptions associated with genetic modification. Striking a balance between innovation and responsible implementation, bolstered by rigorous regulations and international collaboration, is essential to ensure that the Biotech Bounty leads to a future where agriculture thrives sustainably, providing food security for a growing global population while safeguarding our ecosystems.

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