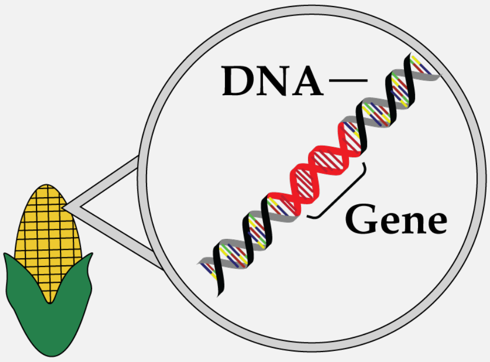
Science & Technology Policy Brief
Genetically Modified Crops



Genetically Modified crops have been commercially cultivated in the world since 1995. This brief outlines the background, techniques of producing GM crops, their potential benefits and associated concerns.

Summary

- Genetic modification (GM) refers to insertion of a gene in an organism.
- GM method can introduce traits from a different organism which is impossible to obtain via conventional breeding.
- Common GM traits in plants are herbicide tolerance (corn), insect resistance (cotton) and virus resistance (papaya).
- Various studies have established that currently available GM crops are safe for human consumption.
- Potential concerns regarding GM crops include impact on biodiversity, soil and genetic diversity among crops.

Background

All living organisms—plants, animals, humans, and bacteria have DNA (Deoxyribonucleic acid). DNA contains the instructions required for life functions such as the development, survival and reproduction of an organism. A gene is a segment of DNA that determines the traits, i.e., features or characteristics inherited from parents. A common example of such a genetic trait is whether a person has straight or curly hair. Sexually-producing organisms have two copies of each gene, one inherited from each parent. The complete set of DNA in an organism is called genome, e.g., the human genome contains around 20,000 genes.[1,2]

Conventional breeding: Conventional or natural breeding has been used for thousands of years to choose specific traits in plants and animals. This selective breeding was done initially without any knowledge of underlying genetics. For instance, different breeds of dogs have been bred for particular traits, e.g., Greyhounds (fast runners) were bred for hunting; Siberian Huskies (possess thick fur coat) were bred to herd reindeers and pull sledges in cold regions. The offspring inherits genes from both parents who have the desired traits. Genetic changes happen over several generations and the breed gets the desired traits. In a way, while selecting desired traits, one ends up selecting a set of genes.

Laboratory-based Conventional Breeding Practices: With recent advances in genetics, conventional breeding also involves a few laboratory-based practices such as *marker-assisted selection* which involves linking markers to genes associated with desirable traits. This technique reduces time and allows the accurate selection of such traits. Another technique called *mutation breeding* has also been used in plant breeding since the 1920s. Mutation refers to changes or variations in genes (DNA sequences). Mutations occur in all organisms at low levels. In this technique, seeds are exposed to radiation (x-rays, gamma rays) and chemicals to increase the rate of mutation to achieve a desired trait. In the 1970s, United States (US) farmers wanted deeper red colouration and sweeter flavour in grapefruits. Scientists achieved it using the mutation breeding technique, and now these varieties make up for most of the grapefruits grown in Texas, US.[3]

Genetic Modification (GM): Modern biotechnological techniques can directly manipulate the gene of an organism. This could be done by modifying the DNA of the organism by inserting genes from the same or another (foreign) species. The introduced gene is integrated into the organism's genome but its location in the DNA is not known. This technique is referred to as genetic engineering and is also often called Genetic Modification (GM). The overall objective is similar to conventional breeding, i.e., to obtain desired traits.

One of the methods of doing genetic modification is to use a biolistic gun (also called a gene gun). Particles of a heavy metal are coated with the gene to be introduced, and then fired with mechanical force into cells, where they get integrated. Another method uses bacteria or virus (containing the gene) to infect the target cells. In both these methods, the

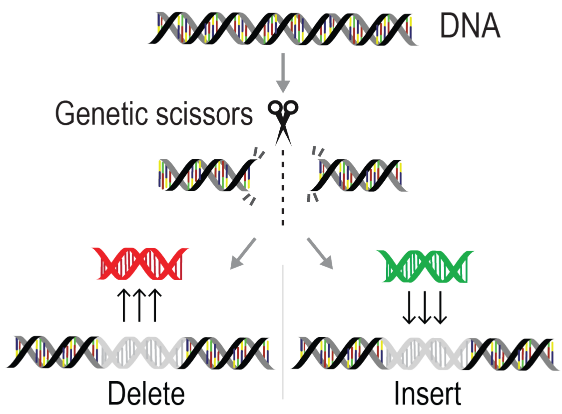
Mayank Shreshtha
mayank@prsindia.org

February 8, 2023

gene is introduced randomly within the target DNA, i.e., its location cannot be specified.

A recently invented technique is *gene editing*, a much more precise form of genetic modification (see Figure 1), which often uses CRISPR technology.[4] It can modify a small fragment of a specific gene at a specific location in an organism's DNA. Examples include gene-edited tomato which helps lower blood pressure and gene-edited soybean oil with enhanced nutritional quality.[5,6]

Figure 1: Gene editing



To summarize, traditional breeding involves selecting traits over many generations, whereas GM technology manipulates the genes directly to obtain those traits.

Benefits of GM technology: GM technology has two major advantages over conventional breeding. First, it is faster to introduce certain traits as conventional breeding requires selection over many generations. Second, it enables changes in genetic makeup that may not be possible through conventional methods, such as the introduction of a gene from a different organism.

The application of GM methods in agricultural plants can result in the following benefits: (i) increased yield, (ii) enhanced yield protection, i.e., resistance to pests and diseases, (iii) reduced costs for food, (iv) reduced usage of environmentally damaging pesticides, (v) enhanced nutritional value, and (vi) tolerance to drought hence reducing the use of groundwater.[7]

Examples where a gene from a foreign organism was introduced in a plant include the following.

Bt-Cotton: The bacterium *Bacillus thuringiensis* (Bt) produces Bt toxin which helps to kill certain pests but is not harmful to humans or other animals. The Bt-toxin genes were introduced in cotton to kill pests if they feed upon it.[7]

Flavr Slavr Tomato: The anti-freezing property from an arctic fish was introduced in tomatoes to increase their shelf life.[8]

Box 1: GM Mustard (DMH-11)

The objective of using GM technology in mustard is to improve yields by crossing an Indian variant (Varuna) with a European line Early Heera (EH)-2. This is difficult to do by conventional breeding because mustard is self-pollinating, i.e., the pollen from the male part pollinates and fertilises the female part of the same plant. This makes it difficult to cross one variant of plant with a different variant.

To overcome this problem, the gene *barnase* is inserted in the Varuna plant which makes it male sterile (no pollen formation).[9,10] This can now be crossed with EH-2. However, the plant growing from the resultant seed will be male-sterile and cannot produce any seed through self-pollination. Therefore, another gene *barstar* which restores male fertility is added to EH-2 before crossing. Thus, the new *barstar-barnase* variant can self-pollinate and produce mustard seeds, which is the desired crop.

As the process of adding the gene is probabilistic (only a few of the target plants get the gene added), another herbicide-tolerant gene *bar* is added along with *barstar* and *barnase*. The resultant (growing) plants are then sprayed with herbicide, and only those containing *bar* gene (and therefore with *barnase* and *barstar*) survive. Therefore, the seeds will have *bar-barnase-barstar* genes. This seed has been named Dhara Mustard Hybrid (DMH)-11.

Common GM traits in plants

GM technology has been used to introduce traits to boost crop production. Some examples are:

Herbicide tolerance (HT): The application of herbicides to kill weeds can damage the crop too. GM crops are created with resistance to specific herbicides which can be used to manage weeds. HT crops reduce soil erosion as weed removal requires ploughing, leading to erosion. They can also be planted in weedy fields. HT crops include corn, cotton and soybeans.

Insect resistance: Crops are created with insecticidal protein which is only harmful to certain pests that feed on them, this removes the need for the external application of chemicals. Bt-crops (cotton and corn) contain such insect-resistant traits.

Virus resistance: Virus-resistant traits are introduced in susceptible plants which do not possess natural resistance. In the 1990s, GM Papaya was created to resist the papaya ringspot virus in Hawaii.[7]

Traits like herbicide tolerance and insect resistance can also be combined, i.e., crops can have multiple traits (e.g., HT-Bt cotton). Other GM traits include aesthetic changes (non-browning Arctic apples) and enhanced nutritional quality (Vitamin A-rich crops such as Golden Rice).

Box 2: GM Regulatory Approaches in the World

United Nations Cartagena Protocol on Biosafety (2000): Signatory countries may take a decision to minimise potential adverse effects on biological diversity and risks to human health with regard to import of living (genetically) modified organisms.[11,12]

USA: If the new variety, made with gene-editing technology could be bred using conventional methods, it does not require regulatory approval (only valid for single modification).[13,14] Regulatory approval focuses on the traits rather than on the technology used.

European Union: Evaluation of risks associated with new GM products is process-based, i.e., it depends on whether or not they were developed using GM methods.[15] Gene-edited crops are also regulated as GM crops.

Canada: All newly developed crops, whether GM or conventionally bred, go through the same risk assessment.

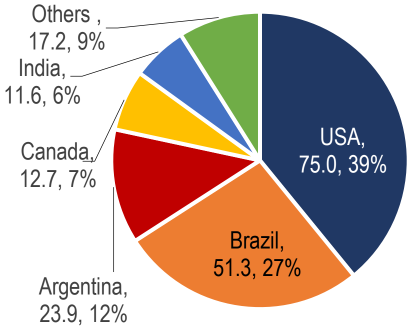
Brazil: The classification of a product as non-GM is based on: (i) absence of foreign gene, (ii) presence of induced mutations which could be achieved by lab-based conventional breeding techniques or via crossing, (iii) presence of naturally-occurring mutations.[16]

Argentina: The main criteria for regulations regarding GM products are: (i) techniques/methods used in the process, (ii) absence of foreign gene in the final product, and (iii) presence of new combinations of genes/DNA sequence(s) in the plant genome.[16] Gene-editing is considered as a non-GM technology and has a separate regulation.[17]

GM Crops in World and India

The introduction of GM crops started with the approval of the Flavr Savr tomato in the USA in 1994.[8] In 2018, GM crops were grown in 26 countries on an estimated 474 million acres (14% of the world's arable land).[18,19] The top GM crops were soybean (50% of area sown of GM crops), maize (31%), cotton (13%), and canola or oilseed rape (5%).[20] These numbers represent 78% of global soybean production, 76% of cotton, and 30% each of maize and canola. USA, Brazil, Argentina, Canada, and India accounted for about 91% of the global GM crop acreage. EU grows only GM maize (mainly in Spain and Portugal).

Figure 2: Global area (in million hectares) of GM crops by country, 2018



Source: International Service for the Acquisition of Agri-biotech Applications (ISAAA); PRS

Box 3: Regulatory Framework in India

Environment Protection Act, 1986: Regulation of GM Crops is primarily governed by "The Manufacture, Use, Import, Export and Storage of Hazardous Micro Organisms Genetically Engineered Organisms or Cells Rules, 1989".[21]

Review Committee on Genetic Manipulation (RCGM): Under the Department of Biotechnology (DBT), this committee monitors various aspects of R & D projects involving GM organisms.[22]

Genetic Engineering Appraisal Committee (GEAC): Under the Ministry of Environment, it is responsible for the assessment of proposals related to the release of GM organisms and products into the environment.[23]

GEAC Safety Assessment tests: Molecular characterisation (study of inserted genes), food safety studies (protein analysis, toxicity and allergenicity tests) and Environmental safety studies (field trials, Biosafety Research Level trials, impact on soil, pollen flow studies).[24,25,26]

GEAC recommendations are considered by Environment Ministry which decides on the final approval of GM organisms and products. In India, gene-edited crops are exempted from biosafety assessment and they will be released as new varieties.[27,28]

GM Crops in India

Bt-Cotton is the only approved GM crop (2002) for commercial cultivation. It was introduced to protect against the widespread infestation of bollworm. In 2018-19, Bt-cotton was 95% of the total cotton planted in India.[18]

Bt-Brinjal: In 2009, Bt-brinjal was cleared by GEAC for commercial cultivation, but it was put on a 10-year moratorium following public backlash and recommendations of brinjal growing states.[29,30] Recently, GEAC has allowed field trials of new varieties of indigenously developed Bt-brinjal in eight states during 2020-23. The trial requires a no objection certificate (NOC) from concerned states and confirmed availability of isolated stretches of agricultural land.[30]

GM Mustard: In October 2022, GEAC approved the environmental release of GM mustard (Dhara Mustard Hybrid/DMH-11, developed in 2002) and its parental crops (Indian and east European lines).[9,10,31,32] The primary objective was to obtain higher yield over its parental lines and other available mustard varieties (see Box 1). It is India's first edible GM crop, and it underwent GEAC regulatory testing from 2008-2016.

GM mustard has not been released for commercial cultivation yet. The approval for environmental release is limited for four years during which it will undergo several post-environmental-release tests (e.g., performance comparison with currently available non-GM variants, effect on honeybees and pollinators).[31] The environmental release of GM mustard has been challenged in the Supreme Court.[33]

Concerns about GM Crops

While allowing GM crops, it may be necessary to ensure that certain adverse consequences do not arise. Some of the important issues include effect on human health and the impact on the environment and biodiversity. However, due to the complexity associated with the long-term assessment of environmental impacts, it may be difficult to reach definite conclusions.[34]

Impact on Human Health

Before any food produced from GM crops is permitted in the market, several safety tests must be completed. Based on decades of data and studies, the scientific community is in agreement that the currently available GM crops are as safe to consume as non-GM crops.[7,34,35,36,37,38]

An example of potential adverse effect of a GM trait on human health comes from Australia. Toxins from beans were put in field peas to kill insects which used to destroy almost 30% of the yield.[39] The feeding trials on animals indicated negative results, and the development of GM field peas was discontinued. Hence, the need for a robust regulatory framework becomes significant.

Impact of herbicide-tolerant and pest-resistant crops

Overuse of herbicides on fields with herbicide-tolerant GM or non-GM crops can allow weeds to develop resistance against them. Glyphosate has been used as a herbicide in the USA since 1974, and its extensive use has led to glyphosate-resistant weeds. Pigweed (*Palmer amaranth)* has recently been found to have developed resistance against six different herbicides (though not when all are applied toegther) in controlled lab experiments.[40] Another example of HT weed is ryegrass in Australia.[41] The unchecked growth of HT weeds can reduce the overall yield.

This issue has been raised in relation to GM Mustard as the process of developing the seed adds a herbicide-tolerant gene (*bar*). The GEAC approval is for use of herbicide at the hybrid seed production stage, and not for the crop.[33] However, the risk remains if farmers use herbicides. The *barnase-barstar* system has been already used to commercially produce HT canola (sister crop of mustard) hybrids in Canada (1995), USA (2001), Japan (2001) and Australia (2002).[42,43,44,45] Some studies on biosafety assessment of herbicide-tolerant *bar* gene indicate that it is safe for human consumption and animal feed, and does not pose any significant risk to environment.[46,47,48]

Impact of herbicide usage on human health

In 2015, WHO - International Agency on the Research for Cancer stated that glyphosate may potentially cause cancer in humans.[49] However, Environmental Protection Agency (USA) and European Food Safety Agency have concluded that glyphosate exposure does not cause cancer.[50,51] Some studies recommend that glyphosate-based herbicides should be subjected to enhanced toxicological tests and scientific investigations during biosafety assessments.[52] Since October 2022, glyphosate usage in India has been restricted except for pest control operators.[53,54]

Impact on soil

Most herbicides degrade quickly in the soil but the rate of degradation depends on soil temperature and moisture levels.[55] The time of application and amount of dosage in recommended limits of herbicides is critical for getting effective results with minimal impact on soil.[56] However, some studies have reported undesired effects of herbicide residue on soil.[57,58]

Impact of pest resistance

Similar to the case of weeds and herbicides, pests can develop resistance to pest-resistant traits such as the Bt toxins produced by the GM crop.

Impact on genetic diversity in crop varieties

Concerns have been raised regarding the potential of GM crops to reduce the genetic diversity of neighbouring crops, close relatives, and weeds.[7,59] Genetic diversity is crucial for adapting to new environments, and as a protection of the species against diseases.

GM crops may crossbreed (physical contact with neighbouring plants, pollen transfer by insects or wind) with non-GM varieties of the same crop and other wild relatives. Such crossbreeding might be problematic if it results in a wild relative crop acquiring unwanted characteristics (weediness, invasiveness).[7] Also weeds could cross breed with HT crops and result in herbicide-tolerant weeds.

Effect on honeybees and other pollinators

There is a concern that GM crops may pose a potential risk to the honeybee population and other pollinators.[60] A few focused scientific studies have not found any direct negative impact on the honeybee population by GM Canola and Bt Crops.[61,62,63] However, HT crops may lead to the overuse of herbicides which may indirectly affect the weed population of an area and thus reduce the availability of pollen or nectar.[60,63] For example, some studies indicate the negative impact of HT crops on the population of the monarch butterfly in the USA; other studies contest these findings and there is no consensus among scientists.[34,57,64]

[1] Willyard, C. (2018). Expanded human gene tally reignites debate. *Nature*, *558*(7710), 354-355, https://www.nature.com/articles/d41586-018-05462-w.

[2] Salzberg, S.L. Open questions: How many genes do we have?. *BMC Biol* 16, 94 (2018), https://doi.org/10.1186/s12915-018-0564-x.

[3] "Delicious Mutant Foods: Mutagenesis and the Genetic Modification Controversy," Genetic Literacy Project (GLP), June 13, 2016, https://geneticliteracyproject.org/2016/06/13/pasta-ruby-grapefruits-why-organic-devotees-love-foods-mutated-by-radiation-and-chemicals.

[4] The Nobel Prize, Popular Science Background: The Nobel Prize in Chemistry 2020, October 7, 2020, https://www.nobelprize.org/uploads/2020/10/popular-chemistryprize2020.pdf.

[5] 'GABA-enriched tomato is first CRISPR-edited food to enter market', *Nature*, December 14, 2021, https://www.nature.com/articles/d41587-021-00026-2.

[6] 'First Commercial Sale of Calyxt High Oleic Soybean Oil on the U.S. Market', Calyxt, February 26, 2019, https://calyxt.com/first-commercial-sale-of-calyxt-high-oleic-soybean-oil-on-the-u-s-market/.

[7] Genetically Modified (GM) Plants: Questions and Answers, The Royal Society, London, 2016, https://royalsociety.org/~/media/policy/projects/gm-plants/gm-plant-q-and-a.pdf.

[8] '1994: Flavr Savr Tomato', National Institute of Health, National Human Genome Research Institute (as accessed on January 3, 2023), https://www.genome.gov/25520336/online-education-kit-1994-flavr-savr-tomato.

[9] Mehra, S., Pareek, A., Bandyopadhyay, P., Sharma, P., Burma, P. K., & Pental, D. (2000). Development of transgenics in Indian oilseed mustard (Brassica juncea) resistant to herbicide phosphinothricin. *Current Science*, *78*(11), 1358–1364, http://repository.ias.ac.in/36413/1/36413.pdf.

[10] Jagannath, Arun et al. "Development of transgenic barstar lines and identification of a male sterile (barnase)/restorer (barstar) combination for heterosis breeding in Indian oilseed mustard (Brassica juncea)." *Current Science* 82 (2002): 46-52, http://repository.ias.ac.in/36263/1/36263.pdf.

[11] Biosafety Brochure: The Cartagena Protocol on Biosafety, Convention on Biological Diversity, https://www.cbd.int/doc/publications/bs-brochure-en.pdf.

[12] 'Text of The Cartagena Protocol on Biosafety', Website of Convention on Biological Diversity (as accessed on January 19, 2023), https://bch.cbd.int/protocol/text/.

[13] Hoffman, N. E. (2021). Revisions to USDA biotechnology regulations: The SECURE rule. *Proceedings of the National Academy of Sciences*, *118*(22), e2004841118, https://www.pnas.org/doi/epdf/10.1073/pnas.2004841118.

[14] Hoffman NE (2022) USDA's revised biotechnology regulation's contribution to increasing agricultural sustainability and responding to climate change. *Front. Plant Sci.* 13:1055529. https://www.frontiersin.org/articles/10.3389/fpls.2022.1055529/full.

[15] Turnbull C, Lillemo M and Hvoslef-Eide TAK (2021) Global Regulation of Genetically Modified Crops Amid the Gene Edited Crop Boom – A Review. *Front. Plant Sci.* 12:630396. https://www.frontiersin.org/articles/10.3389/fpls.2021.630396/full.

[16] Brazilian Agricultural Research Corporation (EMBRAPA), Regulatory framework of genome editing in Brazil and worldwide, 2021, https://www.alice.cnptia.embrapa.br/bitstream/doc/1132164/1/Regulatory-framework-of-genome-CAP-5.pdf.

[17] Friedrichs, S., Takasu, Y., Kearns, P., Dagallier, B., Oshima, R., Schofield, J., & Moreddu, C. (2019). An overview of regulatory approaches to genome editing in agriculture. *Biotechnology Research and Innovation*, *3*(2), 208-220, https://doi.org/10.1016/j.biori.2019.07.001.

[18] ISAAA, Global Status of Commercialised Biotech/GM Crops, Brief No. 54, 2018, https://www.isaaa.org/resources/publications/briefs/54/download/isaaa-brief-54-2018.pdf.

[19] Total arable land (2018) = 3433 million acres, Data portal, Food and Agriculture Organization of the United Nations (FAO), https://www.fao.org/faostat/en/#data/RL.

[20] Table 31, Page 74, ISAAA, Global Status of Commercialised Biotech/GM Crops, Brief No. 54, 2018, https://www.isaaa.org/resources/publications/briefs/54/download/isaaa-brief-54-2018.pdf.

[21] G.S.R. 1037 (E), Ministry of Environment, Forest and Climate Change, December 5, 1989, https://geacindia.gov.in/resource-documents/biosafety-regulations/acts-and-rules/Rules-for-the-manufacture-use-import-export-and-storage-1989.pdf.

[22] 'Review Committee on Genetic Manipulation Procedures', Department of Biotechnology website (as accessed on January 3, 2023), https://ibkp.dbtindia.gov.in/Content/RCGMInfo.

[23] About Genetic Engineering Appraisal Committee (GEAC), GEAC, Ministry of Environment, Forest and Climate Change website (as accessed on January 3, 2023), https://geacindia.gov.in/about-geac-india.aspx.

[24] Pental, D. (2020). Preaching objectivity–practicing obfuscation. *Current Science*, *118*(6), 863. https://www.currentscience.ac.in/Volumes/118/06/0863.pdf.

[25] Guidelines for the Safety Assessment of Foods Derived from Genetically Engineered Plants, GEAC, Ministry of Environment, Forest and Climate Change website, https://geacindia.gov.in/resource-documents/biosafety-regulations/guidelines-and-protocols/Guidelines_for_the_Safety_Assessment_of_Foods.pdf

[26] 'Guidelines and Protocols', GEAC, Ministry of Environment, Forest and Climate Change website (as accessed on January 3, 2023), https://geacindia.gov.in/guidelines-and-protocols.aspx.

[27] Guidelines for Safety Assessment of Genome Edited Plants, 2022, Department of Biotechnology, June 17, 2022, https://dbtindia.gov.in/sites/default/files/Final_%2011052022_Annexure-I%2C%20Genome_Edited_Plants_2022_Hyperlink.pdf.

[28] "Notification of Standard Operating Procedures (SOPs) for regulatory review of Genome Edited Plants under SDN-1 and SDN-2 categories, 2022", Press Information Bureau, Ministry of Science and Technology, October 27, 2022, https://pib.gov.in/PressReleasePage.aspx?PRID=1871153.

[29] 'India's transgenic aubergine in a stew'. *Nature*, February 10, 2010, https://doi.org/10.1038/news.2010.65.

[30] "Study on Cultivation of GM Crops", Press Information Bureau, Ministry of Agriculture & Farmers Welfare, September 15, 2020, https://pib.gov.in/PressReleseDetailm.aspx?PRID=1654492.

[31] Minutes Of The 147th Meeting Of The Genetic Engineering Appraisal Committee, GEAC, October 18, 2022, https://geacindia.gov.in/Uploads/MoMPublished/MoMPublishedOn20221025200345.pdf.

[32] "Secretary, DARE and DG, ICAR Dr Himanshu Pathak today issued a detailed statement on various issues of GM mustard", Press Information Bureau, Ministry of Agriculture & Farmers Welfare, December 23, 2022, https://pib.gov.in/PressReleaseIframePage.aspx?PRID=1886080

[33] Unstarred Question No. 222, Rajya Sabha, Ministry of Environment, Forest and Climate Change, https://pqars.nic.in/annex/258/AU222.pdf.

[34] National Academies of Sciences, Engineering, and Medicine. 2016. *Genetically Engineered Crops: Experiences and Prospects*. Washington, DC: The National Academies Press, https://doi.org/10.17226/23395.

[35] *EASAC Policy Report No. 21,* The Science Advisory Council of the National Science Academies of the EU Member States, 2013, ISBN: 978-3-8047-3181-3, https://easac.eu/fileadmin/PDF_s/reports_statements/Planting_the_Future/EASAC_Planting_the_Future_FULL_REPORT.pdf.

[36] European Commission, Directorate-General for Research and Innovation, (2010). *A decade of EU-funded GMO research*

(2001-2010), Publications Office, https://data.europa.eu/doi/10.2777/97784.

[37] "Food, genetically modified", World Health Organization, May 1, 2014, https://www.who.int/news-room/questions-and-answers/item/food-genetically-modified.

[38] Pellegrino, E., Bedini, S., Nuti, M. *et al.* Impact of genetically engineered maize on agronomic, environmental and toxicological traits: a meta-analysis of 21 years of field data. *Sci Rep* 8, 3113 (2018). https://doi.org/10.1038/s41598-018-21284-2.

[39] "Risk assessment of GM field peas", Commonwealth Scientific and Industrial Research Organisation (CSIRO), December 4, 2015, https://csiropedia.csiro.au/risk-assessment-of-gm-field-peas/.

[40] Shyam C, *et al*. (2021) Predominance of Metabolic Resistance in a Six-Way-Resistant Palmer Amaranth (*Amaranthus palmeri*) Population. *Front. Plant Sci.* 11:614618, https://doi.org/10.3389/fpls.2020.614618.

[41] Bajwa AA, *et al*. The Remarkable Journey of a Weed: Biology and Management of Annual Ryegrass (*Lolium rigidum*) in Conservation Cropping Systems of Australia. *Plants*. 2021; 10(8):1505, https://doi.org/10.3390/plants10081505.

[42] Novel Food Information – Food Biotechnology, Pollination Control System for Canola, MS1/RF1, Health Canada, October 1999, https://www.canada.ca/content/dam/hc-sc/migration/hc-sc/fn-an/alt_formats/hpfb-dgpsa/pdf/gmf-agm/ofb-094-251-a-eng.pdf.

[43] "DD1996-17: Determination of Environmental Safety of Plant Genetic Systems Inc.'s (PGS) Novel Hybridization System for Rapeseed (*Brassica napus* L.)", Canadian Food Inspection Agency, October 1996, https://inspection.canada.ca/plant-varieties/plants-with-novel-traits/approved-under-review/decision-documents/dd1996-17/eng/1303945461567/1303945595359.

[44] Volume 64, No. 61, Federal Register, United States Department of Agriculture, March 31, 1999, https://www.aphis.usda.gov/brs/aphisdocs2/98_27801p_com.pdf

[45] "Event Name: MS1 x RF2 (PGS2)", ISAAA website (as accessed on January 30, 2023), https://www.isaaa.org/gmapprovaldatabase/event/default.asp?EventID=19.

[46] Consensus document on general information concerning the genes and their enzymes that confer tolerance to phosphinothricin herbicide, Report No. 11, ENV/JM/MONO (99)13, Organisation for Economic Co-operation and Development (OECD), June 1999, https://www.oecd.org/env/ehs/biotrack/46815628.pdf.

[47] Module II: Phosphithricin, Report No. 25, ENV/JM/MONO (99)14, OECD, May 3, 2002, https://www.oecd.org/env/ehs/biotrack/46815748.pdf.

[48] Center for Environmental Risk Assessment, ILSI Research Foundation. (2011). A review of the environmental safety of the PAT protein. Environmental Biosafety Research, 10(4), 73-101, https://doi.org/10.1051/ebr/2012004.

[49] "International Agency Research on Cancer (IARC) Monograph on Glyphosate", IARC - World Health Organisation, March 1, 2016, https://www.iarc.who.int/featured-news/media-centre-iarc-news-glyphosate/.

[50] "Glyphosate", US Environmental Protection Agency website (as accessed on December 27, 2022), https://www.epa.gov/ingredients-used-pesticide-products/glyphosate.

[51] "Glyphosate: EFSA updates toxicological profile", European Food Safety Authority, November 12, 2015, https://www.efsa.europa.eu/en/press/news/151112.

[52] Myers, J.P., *et al.* Concerns over use of glyphosate-based herbicides and risks associated with exposures: a consensus statement. *Environ Health* 15, 19 (2016), https://doi.org/10.1186/s12940-016-0117-0.

[53] S.O. 5010 (E), Ministry of Agriculture and Farmers Welfare, October 21, 2022, https://egazette.nic.in/WriteReadData/2022/239860.pdf.

[54] "Ban on use of Glyphosate and other Pesticides", Press Information Bureau, Ministry of Agriculture & Farmers Welfare, December 16, 2022, https://pib.gov.in/PressReleasePage.aspx?PRID=1884235.

[55] "Herbicides", Agriculture and Food Division, Department of Primary Industries and Regional Development, Government of Western Australia, (as accessed on December 28, 2022), https://www.agric.wa.gov.au/herbicides/herbicides?page=0%2C4.

[56] Hagner, M., Mikola, J., Saloniemi, I. *et al.* Effects of a glyphosate-based herbicide on soil animal trophic groups and associated ecosystem functioning in a northern agricultural field. *Sci Rep* **9**, 8540 (2019). https://doi.org/10.1038/s41598-019-44988-5

[57] Schütte, G., V. *et al.* Herbicide resistance and biodiversity: agronomic and environmental aspects of genetically modified herbicide-resistant plants. *Environ Sci Eur* 29, 5 (2017), https://doi.org/10.1186/s12302-016-0100-y.

[58] Helander, M., *et al.* Glyphosate residues in soil affect crop plant germination and growth. *Sci Rep* **9**, 19653 (2019). https://doi.org/10.1038/s41598-019-56195-3

[59] 'Challenging Evolution: How GMOs Can Influence Genetic Diversity', Science In The News, The Graduate School of Arts and Sciences, Harvard University, August 10, 2015, https://sitn.hms.harvard.edu/flash/2015/challenging-evolution-how-gmos-can-influence-genetic-diversity/.

[60] Arpaia, S., Smagghe, G., & Sweet, J. B. (2021). Biosafety of bee pollinators in genetically modified agro-ecosystems: Current approach and further development in the EU. *Pest Management Science*, *77*(6), 2659-2666, https://onlinelibrary.wiley.com/doi/pdfdirect/10.1002/ps.6287.

[61] Huang, Z. Y., *et al.* (2004). Field and semifield evaluation of impacts of transgenic canola pollen on survival and development of worker honey bees. *Journal of economic entomology*, *97*(5), 1517–1523, https://doi.org/10.1603/0022-0493-97.5.1517.

[62] Janet E. Carpenter (2011), Impact of GM crops on biodiversity, GM Crops, 2:1, 7-23, https://www.tandfonline.com/doi/abs/10.4161/gmcr.2.1.15086.

[63] Crops, weeds and pollinators understanding ecological interaction for better management, FAO, 2015, https://www.fao.org/3/i3821e/i3821e.pdf.

[64] John M. Pleasants, *et al.*, Conclusion of No Decline in Summer Monarch Population Not Supported, *Annals of the Entomological Society of America*, Volume 109, Issue 2, March 2016, Pages 169–171, https://doi.org/10.1093/aesa/sav115.