

BIOSAFETY IN THE AGE OF BIOTECHNOLOGY: CHALLENGES AND OPPORTUNITIES WITH GENETICALLY MODIFIED ORGANISMS

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ABSTRACT

Genetically modified organisms (GMOs) have emerged as a product of biotechnological advancements, raising concerns about biosafety and regulatory frameworks. This review explores the intersection of GMO technology and biosafety, covering its evolution, applications as well as regulatory measures. Scientists have used recombinant DNA technology, a mechanism for genetic manipulation to alter organisms. This has significant implications for organismal phenotypes and protein production. GMOs hold significant promise for agriculture, medicine, and industry offering potential benefits for food security and national development. However, concerns remain regarding their environmental impact and human health risks. These concerns include transgene transfer, biodiversity loss, and potential health implications, alongside regulatory frameworks, and risk management strategies. Several international agreements, like the Convention on Biological Diversity and the Cartagena Protocol on Biosafety, aim to regulate GMOs and safeguard biodiversity. Pakistan has implemented Biosafety Rules of 2005 and has established the regulatory bodies like the National Biosafety Committee (NBC). Bacillus thuringiensis (Bt) cotton, a genetically modified crop, is grown in Pakistan. Several studies on food safety and environmental safety have been carried out about BT Cotton's biosafety concerns. This review provides a comprehensive overview of the evolution, applications, risks, and regulatory landscape of GMOs, offering insights into their role in sustainable development and biosafety governance.

Keywords: Genetically modified organisms (GMOs), Biosafety, Cartagena Protocol, National Biosafety Committee (NBC), risk assessment, containment measures

INTRODUCTION

Genetically modified organisms are created through the application of biotechnology enabling scientists to transfer genes from one organism to another through genetic alteration. As a result, the organism develops differently, giving rise to new kinds of plants and animals. Biosafety is the system created through policies and procedures to ensure this application is done in an environmentally safe manner.¹ Through the use of genetic engineering, it is now likely to modify the

genetic makeup of distinct animals, leading to the discovery of novel gene combinations.²

By the use of recombinant DNA technology, a genetically modified organism has had its genetic makeup changed.³ Recombinant DNA technology is the process of linking DNA molecules from numerous sources into one molecule in a test tube. Consequently, changing the genes of an organism permits the altering of the protein production and/or phenotype. The term "test tube" in the context of genetically modified organisms (GMOs) describes the carefully monitored lab settings used to carry out genetic alterations. For gene editing procedures like CRISPR-Cas9 or recombinant DNA approaches, it entails employing test tubes or comparable equipment to ensure accurate alterations and reduce contamination. These techniques make it easier to produce organisms with desired

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characteristics for use in medicine, agriculture, and research.⁴

Genes can be transferred through the manipulation of DNA via genetic engineering. Khan and Ramay in 2013 stated that genetically modified products encompass pharmaceuticals and vaccines, food items and their component parts, feeds and fibers.⁵

Plant breeders in the past have crossed different plant types for altering the genetic composition and introducing the desired features. 'Selective breeding' is the term for this technique.⁶ These days, advancements in research and development have improved our knowledge of the science underlying genes. It is indeed possible for scientists to transfer a single gene from one organism's DNA to another resulting in the desired characteristics. For instance, a plant that can withstand a certain pest or illness, the transfer is also feasible to occur between unrelated species.⁷ GMOs examples include genetically modified agricultural crops that are more productive and resistant to pests or diseases.⁸ Most common GMO crops include soybeans, cotton and maize.² Genetic engineering and modern biotechnology have many uses in industry, agriculture, and medicine. In medical industry the genetically altered *Escherichia coli* produce recombinant insulin. By introducing the human insulin gene into *E. coli*, researchers can make the bacteria produce insulin that is exactly like what is produced in the human body. This method transformed the treatment of diabetes by offering a more scalable and safer substitute for insulin derived from animals.⁹ It's no secret that contemporary biotechnology has great promise for boosting food security, agricultural production, and overall national development especially in developing nations where agriculture plays a major role.² Genetically modified organisms (GMOs) are created when new qualities are incorporated into plants, animals, and microorganisms through the application of genetic engineering in agriculture. These GMOs are subsequently used to cultivate, produce, and manufacture genetically modified foods.

Worldwide in 2019, genetically modified crops were cultivated on 190.4 million hectares land. The main purpose of the added features i.e. genetic modification in the agriculture industry was to keep the crops safe from pests and illnesses. GMOs are anticipated to become widely used as a result of growing acceptance towards GMOs and the development of more varied products. Modern biotechnology has a lot of potential benefits, but there was also a lot of concerns around the world that its products could be harmful to the environment or human health.⁷

People who were more considerate of the environment began to worry that the subsequent GMOs will spread quickly, effecting on-target and endangered species, reduce biodiversity, and other negative effects.¹⁰⁻¹² Some people thought that genetically modified foods (GM foods) made with contemporary biotechnology were completely unique and separate from traditional foods in terms of human health. They should thus be avoided as they had the potential to be hazardous (i.e. poisonous). Concerns about GMOs going beyond safety concerns include the possibility of a seed monopoly, harm to farmers' rights to save seeds and unethical behavior (such as "playing God"), etc.¹³

Animals benefiting from GMOs have higher resistance, productivity, and feed efficiency; they also provide superior meat, egg, and milk yields and have better diagnostic tools and animal health.¹⁴ Better yields, flavor, and quality, shorter maturity times, higher resistance to pests, diseases, and herbicides as well as new products and growing methods are all benefits for crops. Bioprocessing for agricultural products has made natural waste management more effective. This includes 'friendly' bioherbicides and bioinsecticides, initiatives for conserving soil, water, and energy, as well as enhanced food security for expanding populations in society.¹⁵

Concerns about the use of genetically modified organisms were raised by Consulting and Audit Canada for Emergency Preparedness Canada (CACEPC) in 1995. The first category pertains to issues of access and intellectual property. This includes foreign exploitation of natural resources for biopiracy, the dominance of a small number of firms in the world's food supply, and the growing reliance of developing nations on industrialized nations. The second category involves etymological concerns. These include the transgression of inherent values of natural organisms, the manipulation of nature through gene-swapping across species, opposition to eating plant DNA and vice versa, and the stress imposed on animals. In certain nations (like the United States), labeling is not required for certain foods by U.S. Department of Agriculture (USDA) in July 2018, mixing genetically modified and non-GM crops might lead to confusing labeling.

Antibiotic resistance markers, allergies and unknown consequences are some potential health impacts that concerns the use of GMOs. Possible implications on the environment include loss of biodiversity of flora and fauna, unintentional transgene transfer through cross-pollination, and unknown effects on other creatures

(such as soil microorganisms).¹⁶ Recent advancements in GMOs related to society could be biased towards the needs of wealthy nations.

Convention on Biological Diversity (CBD) and Cartagena Protocol on Biosafety

An addition to the Convention on Biological Diversity is an international accord on biosafety called as the Cartagena Protocol on Biosafety. The goal of the Biosafety Protocol is to safeguard biological variety against the possible threats posed by genetically modified organisms. An international agreement known as the Convention on Biological Diversity (CBD) was made at Rio de Janeiro Earth Summit in year 1992. Preserving biological variety, making sustainable use of its constituents and distributing benefits from genetic resources fairly and equally are the three fundamental objectives of the Convention. It became operative on December 29, 1993 after being available for signature on June 5, 1992. Pakistan ratified the Convention on June 5, 1992, and was accepted as a party on July 26, 1994.

The conservation of biological diversity was acknowledged as "a common concern of humankind" and an essential component of the development process for the first time in international law by the agreement. Genetic resources, animals, and ecosystems are all covered under the pact. It connects the financial objective of using biological resources responsibly with conventional conservation initiatives. It establishes guidelines for the just and equal division of gains from the use of genetic resources, particularly those intended for commercial application. Through its Cartagena Protocol on Biosafety, it addresses technological development and transfer, benefit-sharing, and biosafety issues. It also covers the quickly developing sector of biotechnology. A significant feature of the Convention is that its terms are obligatory on the countries who ratify it.¹⁷

A Cartagena Protocol on Biosafety to the Convention on Biodiversity was adopted on January 29, 2000, and it went into effect on September 11, 2003, to adopt adequate measures on trans boundary movements of (live GMOs) LMOs. The Protocol addresses the safe handling, transmission, and application of living modified organisms (LMOs) including transnationally transmissible microorganisms, plants and animals. The goal of the Cartagena Protocol is to prevent negative consequences on biodiversity conservation and sustainable usage while avoiding needless disruptions to the global food trade. As of 2018, 198 nations, including

those in our region like Bangladesh, India, and Iran, had deposited instruments of ratification or accession to the Cartagena Protocol with the UN. Pakistan signed this protocol on June 4th, 2001.¹⁸

Risk Communication, Management and Assessment posed to/by GMOs:

It is generally acknowledged that each nation must set up a regulatory framework expressly to evaluate the safety of modern biotechnology products due to the possible threats that genetically modified organisms may pose to humans and the environment whether actual or perceived.¹⁷ Each nation can select from several choices to investigate the advantages of contemporary biotechnology while also addressing worries about the possible negative impacts of the introduction of genetically modified organisms on the environment and human health. The options pertain to the goals and design of the regulatory system, the means of implementation and regulatory structures, as well as other factors like public involvement, the ability to stand alone or integrate into other national goals, and the ability to be in line with other regional and international commitments.¹⁹

Regardless of the approach chosen by the nation, a biosafety framework usually consists of four key components: a guiding framework, a system for compliance & monitoring, a national biosafety policy tool (such as a decree, act & law) and procedures for guaranteeing accountability, transparency, and public participation.²⁰

GMOs Fate in Pakistan and Role of the Ministry of Environment

In order to fully utilize this cutting-edge technology and ensure the safety of both humans and the environment, Pakistan Biosafety Rules were notified on April 21, 2005. These rules regulate the production, importation, and storage of genetically modified organisms and gene technological products for research purposes, regardless of whether the research is carried out in public or private research and development laboratories or teaching laboratories. The effort covered the development of genetically modified organisms for use in plants, animals, and microorganisms, as well as their commercial release into the field and their import, export, sale and purchase.

Following the publication of the Biosafety Rules in 2005, the National Biosafety Guidelines in 2005 were created. These guidelines specify the appropriate

protocol and documentation needed to conduct the aforementioned GMO-related activities within the safety parameters. Legal protection for the National Biosafety Guidelines and their execution in the nation is provided by the Pakistan Biosafety Rules 2005.

Three tiers, National Biosafety Committee (NBC), Technical Advisory Committee (TAC), and Institutional Biosafety Committee (IBC), are the foundation of the framework for overseeing and putting the National Biosafety Guidelines into practice, as outlined in the Biosafety Rules, 2005. Overseeing all laboratory work, field trials, commercial release, import, export, sale, and procurement of genetically modified organisms and their products, NBC is led by the Secretary of the Ministry of the Environment. According to the National Biosafety Guidelines 2005, all applications and requests for any kind of GMO-related activity must be presented to the appropriate IBC, which serves as the baseline regulatory, implementing and monitoring body. These must then be given to TAC for evaluation, and NBC will take any additional required action based on its recommendations.²¹

Guideline for work with genetically modified microorganisms

When working with genetically modified microorganisms, initial evaluation of the nature of the biological system is required for microorganisms that have previously been used safely in the field. Microorganisms from a strain proven to perform the same functions as those used in previous documented field studies are considered suitable. These microorganisms must be limited to locations and environments similar to the earlier studies and have a proven history of safe use.²²

As stated in the Regulations and Containment Section, the study may continue with appropriate containment levels for experimental microorganisms that do not meet the previously indicated requirements. Microbes are contained appropriately biologically, meaning that before being field tested, they are rendered non-reproducible; alternatively, modifications are made to restrict the amount of time that microorganisms can survive outside and confine them within target areas; recombinant DNA techniques are applied to microorganisms only within designated areas; and physical measures are taken to prevent microorganism dispersal within the target areas or trial site.²³

For microorganisms that have not previously been used safely in the field, a preliminary risk assessment may be

performed to evaluate the complete spectrum of potential environmental consequences. Biological control of plant pests can overpower target species and produce harmful toxins or pathogens. It may leave toxic residues with secondary negative effects or spread diseases in wild populations. Excess nutrient supply from controlled plants can disrupt the chemistry of nearby plants which should be monitored.²⁴

Guideline for work with genetically modified plants

When working with genetically modified plants in the field, it is important to first analyze the biological systems' nature. Work may continue in compliance with the fundamental guidelines suitable for the specific plant in question if experimental plants are thought to have a history of safe field use. The organism must be modified through standard breeding techniques, showing unique traits that distinguish it from traditionally bred plants. The introduced genetic material should also be safe and not harmful to the environment.²⁵

If experimental plants don't fit the above requirements, work can still be done as long as the right containment level and standards are followed. There must be no cross-hybridization, plans in place to restrict the spread of plants and plant materials, and introduced gene expression that is stable and does not change in response to environmental changes, among other requirements, for the aforementioned containment measures to be effective.⁹

For the plants that have not previously been used safely in the field, work may begin with a preliminary risk assessment to determine the effects on the experiment site's ecology: enhanced resilience to diseases and pests, proclivity for weeding, and effects on other targets and non-target organisms. Effects on open-air ecology, possibility for cross-hybridization, weed promotion and stimulation, invasion of natural populations beyond the trial site, and effects on other factors in the environment may also be addressed.²⁴

Guideline for work with genetically modified animals

The following guidelines should be followed while breeding genetically engineered animals: Identifiable containers should be used to breed non-engineered animals, and genetically modified animals should be kept separate from other animals. Animals modified through genetic engineering should be housed and identified individually. After sterilization and burning if required, wastes associated with genetically modified animals should be disposed of. GMO animals should be

transported outside of the work area in containers that are strong and well-constructed to keep them from escaping. Genetically engineered animal containers need to be handled carefully, and this needs to be mentioned. Performance testing should be conducted on the facilities, equipment, and management systems used in these investigations.²⁶

At every work area, a sign identifying genetically modified animals should be displayed. Additionally, the area must be kept clean, personnel should only wear work clothes there, and the person in charge must inform receiving personnel of all pertinent information when transferring genetically modified animals to other facilities.^{24,27}

Regulation and containment for experimental genetically modified microorganism

Having Past Field Experience: To conduct field testing on microorganisms that have previously undergone fieldwork, a project proposal must be submitted to the IBC. This proposal will assess the adequacy of biosafety protocols. Regulatory compliance and the specific microorganisms being studied must be taken into consideration while designing containment and control measures for fieldwork. Only after gaining an IBC endorsement can the work start. All assessments and recommendations must be sent by the IBC to the NBC via the TAC to obtain documentation and information.²⁸

Without Past Field Work Experience: Field testing experimental microorganisms without previous fieldwork experience should be carried out with guidance and approval from the IBC and NBC. Approvals in these situations will be determined by the biosafety risks that may be discovered from the submitted written proposals. Before receiving approval from the NBC, the project supervisor is not allowed to start any work.

Since untested experimental microorganisms carry dangers, the following needs to be considered when designing procedures for field trial control and containment: The NBC has approved the levels of regulation and containment for the testing medium, which includes soil, water and air used for microorganism analysis. A clear demarcation and posting of the boundaries are required for testing zones. "No Entry" placards. Testing locations are used under tight regulations. An NBC-approved method of close observation and efficacy is used to track the spread of experimental microorganisms. At the end of the project, plans are established to eliminate or deactivate the

experimental microbes. Additional actions that the IBC or NBC judge appropriate.²⁴

Regulation and containment for experimental genetically modified Plants

Despite previous field work experience, experimental plant field testing still involves submitting a proposal to the IBC that will assess the effectiveness of the biosafety measures. This proposal can be submitted at home or abroad. The applicable regulations should be followed while implementing measures to contain and manage field work. In certain cases, work may commence only after gaining an IBC endorsement. The IBC must transmit all suggestions and the committees' assessments, to the National Biosafety Committee through concerned ministries for records and information.²⁸

IBC and the relevant ministry should provide advice, counsel and direction before field testing experimental plants that have never been subjected to previous field operations. Permissions in these situations will be granted based on any biosafety information gleaned from the submitted written proposals. It is forbidden for the project manager to start working until the NBC gives their approval.²⁵

The scale and duration of contained cultivation is appropriate to both the nature of the investigation and the particular plant, and measures for the control and containment of field trials must account for the following, given the risks associated with using untested experimental plants: contained tests may be carried out in plant glass houses. For the specific plant being studied, the selected location is appropriate. Isolated from feral populations, test plots are fenced in. Along the perimeter, there are "No Entry" signs posted regularly. When work is over, plans are made to gather, burn, and destroy experimental plants and plant materials.⁹

The IBC regularly surveys and directs plant cultivation in accordance with the growth or developmental trends of each individual plant.^{28,29}

Regulation and containment for experimental genetically modified animals

After gaining IBC approval, genetically modified animals with a history of previous field experience, whether domestically or overseas, may undertake field trials. For records and information, the IBC is required to forward all recommendations and the committee's evaluation to the National Biosafety Committee (NBC) via the relevant ministry. Under the supervision, advice,

and guidance of IBC and the relevant ministry, field testing of experimental animals that have never been utilized for field research should start. The project manager is not allowed to start working before the NBC gives its approval. Field trials must be controlled and contained at all times, considering potential dangers related to the use of genetically modified animals.^{26,28,30}

Implementation of Project “National Biosafety Centre”

The Secretariat of the National Biosafety Committee is the National Biosafety Centre. The necessary infrastructure for putting the 2005 Biosafety Guidelines and Rules into effect is provided by the National Biosafety Centre. Protecting people against the unfavorable effects of genetically modified organisms is the center's main goal. Public notifications, notices, workshops, and seminars may be organized nationwide on the federal and provincial levels to increase awareness about GMOs and the risks they pose to human health and the environment.^{31,32}

A Case study

Patent GMO, Bt Cotton, which has a toxin gene from a *Bacillus* species added to it, is genetically engineered in Pakistan. Several studies on food safety and environmental safety have been carried out about Bt Cotton's biosafety concerns.^{33,34} Research on the security of the environment pollen escape revealed that the pollen travel is restricted because of precautionary measures; there is no possibility of transfer from cultured diploid species to tetraploid Bt hybrids, now the wild species of Bt cotton are nonexistent. The study found no significant variations in germination and vigor between Bt and non-Bt cotton. Therefore, there is no discernible difference between the two types of cotton in terms of weediness and aggression potential.

Impact of Bt on non-target organisms

On non-target species like sucking pests (aphids, whiteflies, and mites), cotton hybrids had no toxic effects. Both Bt and non-Bt cotton hybrids showed the continued activity of beneficial insects such as ladybirds, beetles, honeybees, and spiders. Bt protein found in soil, indicate that the Cry1Ac protein was broken down quickly in the soil, protein was not found in soil samples. According to calculations, the half-life of the Cry1Ac protein in plant tissues is 41 days, which is similar to the rates of degradation observed for Bt

microbial formulations. The impact of Bt protein on soil microflora was determined by comparing the populations of microorganisms and soil invertebrates, such as earthworms, in Bt and non-Bt samples. The results indicated no discernible differences in these populations. Bt protein impact on soil microflora between Bt and non-Bt samples revealed no appreciable variation in the population of microorganisms and soil invertebrates, such as earthworms.^{9,35}

Conference

In conclusion, a strong regulatory framework and extensive biosafety procedures are needed for the production and use of genetically modified organisms to mitigate any potential dangers. While there are advantages to GMOs, such as increased food security and agricultural production, concerns over their effects on the environment and public health are legitimate. Nations like Pakistan have regulated the manufacture, import, and use of genetically modified organisms by establishing rules and supervision systems such as the National Biosafety Guidelines (2005) and the Pakistan Biosafety Rules (2005) to achieve a balance. In conjunction with the National Biosafety Centre, this multi-tiered system of committees seeks to safeguard the environment and human health while promoting the appropriate use of genetically modified organisms. Ultimately, a comprehensive approach that considers both the potential benefits and the legitimate concerns surrounding GMOs is essential for their safe and sustainable deployment.

Future Recommendations

In future, ongoing assessments of the long-term effects of genetically modified organisms on the environment and human health to inform regulatory actions, should be made. Expanding the global collaboration and standardize biosafety frameworks to promote information exchange and standardized risk assessment. Developing public involvement and awareness campaigns to help people better grasp the advantages and disadvantages of genetically modified organisms. Encouraging the study of substitute sustainable agriculture methods to expand the range of options available to replace GMOs.

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REFERENCE

1. Belgian Biosafety Server. International classification schemes for micro-organisms based on their biological risks. 2006 URL: <http://www.biosafety.be/RA/Class/ClassINT.html>
2. Ranawat B, Shah F, Dubey S, Singh A, Mishra S. Emerging biotechnologies in agriculture for efficient farming and global food production. In *Biotechnological innovations for environmental bioremediation* 2022 Aug 4 (pp. 353-369). Singapore: Springer Nature Singapore. doi: 10.1007/978-981-16-9001-3_14
3. Griffiths AJF., Doebley J., Peichel C., Wassarman DA. An Introduction to Genetic Analysis 2-36. W.H. Freeman and Company; New York, NY, USA: 2020. www.scribd.com/document/673161469
4. Amarakoon, I.I., Hamilton, C.L., Mitchell, S.A., Tennant, P.F. and Roye, M.E., 2024. Biotechnology: principles and applications. In *Pharmacognosy* (pp. 627-645). Academic Press. doi:10.1016/B978-0-443-18657-8.00017-7
5. Khan IA. & Ramay R. Genetic engineering & biotechnology. M. I. Publisher Pakistan, 2013:244-255. <https://www.paspk.org/wp-content/uploads/2015/12/Biotechnology-Report-2014.pdf>
6. Wieczorek, A. M. and Wright, M. G. History of agricultural biotechnology: How crop development has evolved. *Nature Education Knowledge* 2012, 3(10):9 <https://www.nature.com/scitable/25885295>
7. Liu, D., 2024. From Past Achievements to Future Breakthroughs. *Handbook of Molecular Biotechnology*, p.1.
8. Yali W. Application of Genetically Modified Organism (GMO) crop technology and its implications in modern agriculture. *International journal of agricultural science and food technology*. 2022 Jan 8;8(1):014-20. doi: 10.17352/2455-815X.000139
9. Baeshen NA, Baeshen MN, Sheikh A, Bora RS, Ahmed MM, Ramadan HA, Saini KS, Redwan EM. Cell factories for insulin production. *Microbial cell factories*. 2014 Dec; 13:1-9. doi: 10.1186/s12934-014-0141-0
10. Babar U, Nawaz MA, Arshad U, Azhar MT, Atif RM, Golokhvast KS, Tsatsakis AM, Shcherbakova K, Chung G, Rana IA. Transgenic crops for the agricultural improvement in Pakistan: a perspective of environmental stresses and the current status of genetically modified crops. *GM crops & food*. 2020 Jan 2;11(1):1-29. doi: 10.1080/21645698.2019.1680078
11. Prakash D, Verma S, Bhatia R, Tiwary BN. Risks and precautions of genetically modified organisms. *International Scholarly Research Notices*. 2011;2011(1):369573. doi:10.5402/2011/369573
12. Gabol WA, Ahmed A, Bux H, Ahmed K, Mahar A, Laghari S. Genetically modified organisms (GMOs) in Pakistan. *African Journal of Biotechnology*. 2012 Feb 9;11(12):2807-13. doi:10.5897/AJBX11.001
13. Morais PP, Borém A. GM Cultivars. In: Lopes da Silva, F., Borém, A., Sediyaama, T., Ludke, W. (eds) *Soybean Breeding*. Springer, Cham. 2017:311-27. doi: 10.1007/978-3-319-57433-2_16
14. Houdebine LM. Impacts of genetically modified animals on the ecosystem and human activities. *Global Bioethics*. 2014 Jan 2;25(1):3-18. doi: 10.1080/11287462.2014.894709
15. Usmani Z, Sharma M, Awasthi AK, Sivakumar N, Lukk T, Pecoraro L, Thakur VK, Roberts D, Newbold J, Gupta VK. Bioprocessing of waste biomass for sustainable product development and minimizing environmental impact. *Bioresource Technology*. 2021 Feb 1; 322:124548. doi: 10.1016/j.biortech.2020.124548
16. Layton R, Smith J, Macdonald P, Letchumanan R, Keese P, Lema M. Building better environmental risk assessments. *Frontiers in bioengineering and biotechnology*. 2015 Aug 6; 3:110. doi: 10.3389/fbioe.2015.00110
17. Lachenmeier DW, Montagnon C. Convention on Biological Diversity (CBD) and the Nagoya Protocol: Implications and Compliance Strategies for the Global Coffee Community. *Foods*. 2024 Jan 13;13(2):254. doi: 10.3390/foods13020254
18. Tsatsakis AM, Nawaz MA, Kouretas D, Balias G, Savolainen K, Tutelyan VA, Golokhvast KS, Lee

- JD, Yang SH, Chung G. Environmental impacts of genetically modified plants: a review. *Environmental research*. 2017 Jul 1; 156:818-33. doi: 10.1016/j.envres.2017.03.011
19. Eckerstorfer MF, Engelhard M, Heissenberger A, Simon S, Teichmann H. Plants developed by new genetic modification techniques—comparison of existing regulatory frameworks in the EU and non-EU countries. *Frontiers in Bioengineering and Biotechnology*. 2019 Feb 19; 7:26. doi: 10.3389/fbioe.2019.00026
 20. Ghag, S.B., 2024. Genetically modified organisms and their regulatory frameworks. In *Global Regulatory Outlook for CRISPRized Plants* (pp. 147-166). Academic Press. doi: 10.1016/B978-0-443-18444-4.00023-5
 21. Choi IY, Um T, Chung G. Genetically modified organisms in Korea: state of affairs, policy, and regulation. In *GMOs and Political Stance 2023* Jan 1 (pp. 115-127). Academic Press. doi: 10.1016/B978-0-12-823903-2.00011-1
 22. Plavec TV, Berlec A. Safety aspects of genetically modified lactic acid bacteria. *Microorganisms*. 2020 Feb 21;8(2):297. doi: 10.3390/microorganisms 8020297
 23. EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (EFSA CEP Panel), Lambré C, Barat Baviera JM, Bolognesi C, Cocconcelli PS, Crebelli R, Gott DM, Grob K, Lampi E, Mengelers M, Mortensen A. Scientific Guidance for the submission of dossiers on Food Enzymes. *EFSA Journal*. 2021 Oct;19(10): e06851. doi: 10.2903/j.efsa.2021.6851
 24. Authority EF. EFSA statement on the requirements for whole genome sequence analysis of microorganisms intentionally used in the food chain. *EFSA Journal*. 2024 Aug;22(8). doi:10.2903/j.efsa.2021.6506
 25. Raza SH, Zaman U, Ferreira P, Fariás P. An Experimental evidence on public acceptance of genetically modified food through advertisement framing on health and environmental benefits, objective knowledge, and risk reduction. *International Journal of Environmental Research and Public Health*. 2021 May 15;18(10):5264. doi: 10.3390/ijerph18105264
 26. Safdar M, Ullah M, Bibi A, Khan MA, Rehman M, Fatima Z, Hussain M, Awan UA, Naeem M. The Evolving Landscape of Biosafety and Biosecurity: A Review of International Guidelines and Best Practices. *Journal of Women Medical and Dental College*. 2023 Dec 13;2(2). doi: 10.56600/jwmde.v2i2.73
 27. National biosafety guideline. Guidelines for field work. Pakistan environmental protection agency, 2005a:16-18.
 28. Awais M, Rao AQ, Sadaqat S, Yaqoob A, Bhutta MS, Shakoor S, Yasmeen A, Ajmal S, Ghramh HA, Khan KA, Bakhsh A. The regulatory landscape of genetically engineered crops in Pakistan. *Food and Energy Security*. 2024 Jan;13(1): e494. doi:10.1002/fes3.494
 29. World Health Organization. Surveillance of congenital anomalies: a quick reference manual for congenital anomalies and selected infections. World Health Organization; 2022 Aug 31. 9789240015418
 30. National biosafety guideline. Guidelines for field work. Pakistan environmental protection agency, 2005b: 19-20.
 31. Sheppard AW, Hill R, DeClerck-Floate RA, McClay A, Olckers T, Quimby PJ, Zimmermann HG. A global review of risk-benefit-cost analysis for the introduction of classical biological control agents against weeds: a crisis in the making? 2003: 91-108. www.researchgate.net/publication/279886253_A
 32. Wiley LF. Climate change adaptation and public health law. In *Research Handbook on Climate Change Adaptation Law 2013* Jul 30 (pp. 171-213). Edward Elgar Publishing. doi: 10.4337/9781800371491.00012
 33. Govil OP. Biosafety Issues and Bt cotton—A case study. 2012; Professor Genetics IARI. www.scribd.com/document/117936862
 34. Glick BR, Patten CL. *Molecular biotechnology: principles and applications of recombinant DNA*. John Wiley & Sons; 2022 Mar 15. ISBN: 978-1-683-67366-8
 35. Romeis J, Meissle M, Bigler F. Transgenic crops expressing *Bacillus thuringiensis* toxins and biological control. *Nature biotechnology*. 2006 Jan 1;24(1):63-71. doi: 10.1038/nbt1180