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RESEARCH PAPER

TITLE

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Abstract

Genetically modified organisms (GMOs) are organisms whose genetic material (DNA) has been artificially modified by using genetic engineering techniques to enhance and altered their characteristics. Genetic engineering plays a significant role in the development of transgenic crops. The four crops canola, maize, cotton, and soybean are the most common ones to use GM crop technology, which has been used extensively for more than 20 years in a variety of nations. The two most significant GM crops in Pakistan are cotton and maize, both of which are resistant to weeds and insects. The impact studies of insect-resistant and herbicide-tolerant crops that are already available demonstrate the advantages of these techniques for both producers and consumers, as well as their favorable effects on both the environment and public health. Additionally, GM crops are a treatment for famine and malnutrition. Future uses may perhaps provide even greater benefits. Implementing an integrated strategy to pest management will be essential for food security, agricultural stability, and protection of the environment as the global population increases. Crops that have been genetically modified (GE) offer resistance to herbicides or protection from pests and diseases. Technology significantly decreases pest damage and improves crop production. As in the case of Bt cotton, pest-resistant genetically modified crops can support higher yields and agricultural development. We provide a thorough update on the status of the cultivated genetically modified (GM) crops in this paper. We address some vector based techniques for modification and some new approaches of transgene transfer without microbial vector insertion into recipient species In order to reduce the hazards associated with microbial vectors.

1. Introduction:

Genetically modified organisms (GMOs) are organisms whose genetic material (DNA) has been purposely altered via the use of genetic engineering techniques in order to improve and change their traits. Since 1970, the development of contemporary biotechnology has made it feasible to transfer genetic material across animals that are homologous and those that are not(Saxena, Kishor et al. 2020). It is crucial to ensure that these modified organisms or their byproducts do not negatively affect the environment or human welfare when released into nature (Zhang, Wohlhueter et al. 2016). Genetically modified crops are those that have had their DNA altered utilising genetic engineering techniques (GM crops). Transgenic crops (GM crops) are developed via genetic engineering to include a number of features that are not seen in naturally occurring organisms(Kamle, Kumar et al. 2017). For instance, GM crops are produced when a desired gene is extracted, cloned, and put into the desired crop in order to give disease and pest resistance, increased yield, and the generation of new and original features (Kamthan, Chaudhuri et al. 2016).

Physical methods or the employment of Agrobacterium to transmit sequences encoded in T-DNA binary vectors can be used to edit plant genomes. In most situations, the goal is to endow the plant with a new characteristic that does not exist in the species naturally.

Because of this, modified food crops have improved nutritional profiles, decreased spoilage, and resistance to chemical treatments (such as herbicide resistance) in addition to resistance to some pests, diseases, and environmental factors(Sharma, Bhatnagar-Mathur et al. 2005). The intention is to give the plant a new trait that doesn't occur in the species normally. The main obstacle to changing the genes of desirable characteristics in plants and crops is the choice of vectors. Microbial vectors are the most often utilised kind (bacterial and viral). Crown gall disease is brought on by the naturally occurring soil bacterium ***Agrobacterium tumefaciens***. It injects a fragment of its own DNA into the plant cell when it infects a host. The desired gene to be transferred is incorporated into its DNA, and genetic material change occurs in the appropriate plants and crops. like Bt crops. Genetically modified crops, sometimes referred to as GMOs, are produced using this technique and are nutritionally altered as well as insect- or herbicide-resistant. The first Bt crop, tobacco, was grown in 1985, but it was not a commercial success. Bt crops (corn, cotton, potato, and tobacco) were able to be produced and sold starting in 1995(Kamle, Kumar et al. 2017). The most common Bt crops right now are corn and cotton(Abbas 2018). Genetically modified (GM) crops are seeds that have been produced more abundantly using biotechnology. These seeds have certain defence systems that enable them to resist pesticides, diseases, viruses, and herbicides. Two examples of first-generation GM include insect-resistant maize and herbicide-resistant (glyphosate) soybeans crops(Raman 2017). The second generation has a direct connection to crops that have novel traits that are valuable to consumers and boost protein levels(Rocha-Munive, Soberón et al. 2018). Some GM crops include rice with added beta-carotene or minerals, tomato with enhanced carotenoids, maize with higher vitamin C levels, soybean with improved amino acid composition, and potato with more calcium(Raman 2017). Third-generation GM plants are now going through the scientific review procedure. Some of the genetic modifications made to these plants are meant to boost their output by strengthening their ability to withstand environmental stress. Other GE crops generate food with enhanced health benefits or from renewable basic materials. Agricultural biotechnology's advancement has presented societal and ethical difficulties. Most biotech crops available today have been genetically altered to express certain features that are advantageous to people, plants, and the environment(Singh 2021). ' For the purpose of improving agricultural cultivars, new technologies are being employed to create novel alterations in genes. These entail producing and expressing desired features for certain chromosomes by chromosomal modification(Phillips 2008). Despite possible advantages, various hazards and worries about the environment and food safety of GM crops have been noted, including the creation of allergens and the rising global problem of antibiotic resistance gene(Mueller 2019). The natural protein output of plants is altered when microbial vectors are used to modify crops and plants. Integration and interference in the metabolic pathway of plants and crops occur as genes are transported through the vector (microbe)(Dizon, Costa et al. 2016). This happens as a result of integrated gene sequence loss, gene instability, and disruption of gene function. While consuming GM foods, these circumstances result in the formation of allergens, poisons, and various illnesses like cancer, diabetes, liver toxicity, and kidney toxicity. One such is GM maize kernels, which release the gut toxic and poorly digestible Bt toxin Cry1F(Ribeiro, Arraes et al. 2017).

The usage of genetically modified organisms (GMOs) has significantly increased recently on a global scale. 2011 saw the planting of 160 million hectares of GM crops, making them the most widely used agricultural technology in the annals of modern agriculture(Jouzani 2012). Most farmers have adopted GM Technology. 53.6 million acres of GM maize were being grown in 2015, making up about one-third of the total corn crop. The yield was greater by 5.6 to 24.5 percent and contained less mycotoxins(Koch, Ward et al. 2015). Pesticide use decreased by 37% in 2018, crop yields increased by 22%, and farmer profits increased by 68%. The ecosystem has benefited from this decrease in pesticide use(Brookes and Barfoot 2018). There is scientific proof that food made from GM crops does not pose a greater threat to human health than traditional food, but GM food has to be tested side by side with conventional food(Kamle, Kumar et al. 2017). The general population is less likely than experts to think that foods containing genetic modifications are safe. Officially, they are prohibited in 38 countries, including 19 in Europe(Eckerstorfer, Engelhard et al. 2019).

2. History

Southwest Asia, where emmer and einkorn wheat were grown, is where the first signs of plant domestication were found. Enzymes that allowed DNA to be sliced at specific locations were discovered in the 1970s. After being discovered in 1952, plasmids immediately emerged as crucial tools for transferring genetic material across cells(Glass-O'Shea 2011). According to the WHO, Arabidopsis thaliana produced genetically altered seeds in 2008 by soaking flowers in Agrobacterium. Tobacco was the first agricultural plant to be genetically modified, according to accounts from 1983. It was created using a chimeric gene that joined an Agrobacterium T1 plasmid with an antibiotic-resistance gene(Chen and Otten 2017). The chimeric gene was introduced into the plant when tobacco was infected with Agrobacterium and transformed with this plasmid. A new plant was produced using tissue culture techniques from a single tobacco cell that was expressing the gene(Zhang, Wohlhueter et al. 2016). In 1986, tobacco plants were used in field tests of genetically altered plants in France and the United States. The first business to genetically create insect-resistant plants with insecticidal protein-producing genes was Plant Genetic Systems, which was established in 1987 by Marc Van Montagu and Jeff Schell(Glass-O'Shea 2011). The number of field testing exposures authorised by the USDA increased from 4 in 1985 to 1,194 in 2002, with an average of around 800 per year. Both the number of locations per release and the quantity of gene constructions—the techniques by which a foreign gene is packed with extra features—have increased significantly since 2005(Kamle, Kumar et al. 2017). From 1,043 in 2005 to 5,190 in 2013(Zimny, Sowa et al. 2019), more agriculturally beneficial GM products, such as drought-resistant crops, were released. Around 7,800 maize releases, 2,200 soybean releases, 1,100 cotton releases, and roughly 900 potato innovations had all been approved as of September 2013(Zakir and Alemayehu). Herbicide tolerance, insect resistance, enhanced nutritional status, and agronomic features like drought resistance are all permitted in the modified crops (GM crops) created using transformation methods.

Methodology of GM crops modification:

The transgene is introduced by genetic transformation. For agricultural productivity and genetic advancement, it has grown to be a vital tool(Low, Yang et al. 2018). Numerous steps are necessary for the genetic transformation process, including the choice and identification of the target gene (transgene), isolation from the host species, and cloning into a particular plasmid carrier(Nazir and Iqbal 2019). Antibiotic and visual selection cues are employed(Ebinuma, Sugita et al. 1997). ***Agrobacterium tumefaciens'*** silenced Ti-plasmid is one example of a biological vector that can be used to introduce a transgene into plant tissue. The final expression and integration of the transgene into plant tissue is typically achieved using one of two methods: either a direct DNA delivery system, such as a biolistic gene gun, or a biological vector. Both methods have been used effectively to introduce transgenes into plants(Barampuram and Zhang 2011);(Baltes, Gil-Humanes et al. 2017)(ii) gene introduction by biological vectors such as a silenced Ti-plasmid from Agrobacterium tumefaciens. Both approaches have successfully been employed for the introduction of transgenes in plants(Gelvin 2003). Other methods such as **electroporation and microinjection** are also used for modification purpose(Council 2004). Recent innovations CRISP and TALEN provide precise and practical editing techniques(Nemudryi, Valetdinova et al. 2014).

Vector based techniques of modification:

Crown gall disease is brought on by a naturally existing soil bacterium called ***Agrobacterium tumefaciens***. The transplanted DNA gets incorporated into the plant's DNA, allowing the plant to read and use the transferred genes much like its own(Hwang, Yu et al. 2017). The transfer results in the production of several distinctive properties. By acting as a vector, the bacterium enables the introduction of foreign genes into plants(Kumar and Ling 2021). Agrobacterium strains with disease-causing genes removed were created in the early 1980s(Hwang, Yu et al. 2017), but they could still attach to and transfer DNA from susceptible plant cells. Researchers have created novel Agrobacterium strains that successfully transfer and integrate specific genetic material into the cells of the target plant species by substituting the DNA of the disease-causing crown gall that is of interest(Nester 2015). Agrobacterium, a naturally occurring genetic engineering agent, produces around 90% of GE crops(Gelvin 2003). Almost 90% of GE crops are produced by Agrobacterium, a naturally occurring genetic engineering agent(Nester 2015). For dicotyledonous plants, such as potatoes, tomatoes, and tobacco, this technique is particularly advantageous(Sankari, Rao et al. 2018). Agrobacteria infection is less effective in crops like wheat and maize(Singh and Kumar 2022).

Bt crops

The endospore (or crystal) form of the Bt poisons is incorporated into most Bt crops, which are thought to be insect pest-resistant plants(Gu, Ye et al. 2021). The first Bt crop (tobacco) was created in a lab by "Plant Genetic Systems" in 1985; however, the crop was never commercialised. In 1995, the Environmental Protection Agency (EPA) in the US gave its approval for Bt crops to be produced and sold commercially. (Cotton, potato, tobacco, and corn)(Abbas 2018). The two most often used BT crops are corn and cotton. BT crops are plants that have been genetically modified to carry the Bt toxin and are grown on 100 million acres(Koch, Ward et al. 2015). The usage of BT cotton has resulted in a considerable decrease in the number of target pests in cotton as well as other crops. Furthermore, the reduced use of pesticides enabled pest management activities in BT agricultural fields(Naranjo 2011).

Diagram

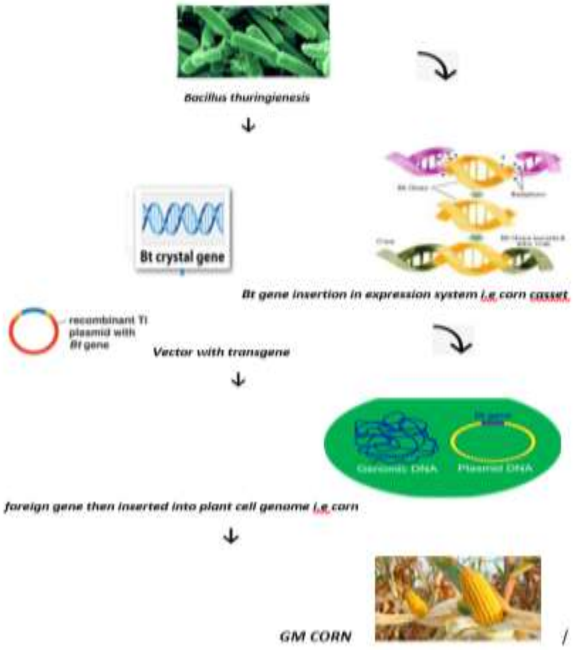

Diagram illustrating Bt-mediated transformation

2.1. Gene gun

Gene guns, commonly referred to as biolistic weapons, "insert" radiation or energy particles right into plant cells. Small gold or tungsten particles that have been coupled with DNA are fired at high pressure within plant cells. The membranes and cell walls are both penetrated by the accelerated particles. Plant DNA is inserted with the metal-isolated DNA that was found inside the nucleus(Baltes, Gil-Humanes et al. 2017). Many farmed crops have been successfully transformed using this technology, although it has been less effective for major crops with single seeds, like wheat or maize. This therapy's disadvantage is that it might seriously harm biological tissue. It is the method of genetic engineering that is most frequently used(Bhatia, Sharma et al. 2015).

Diagram

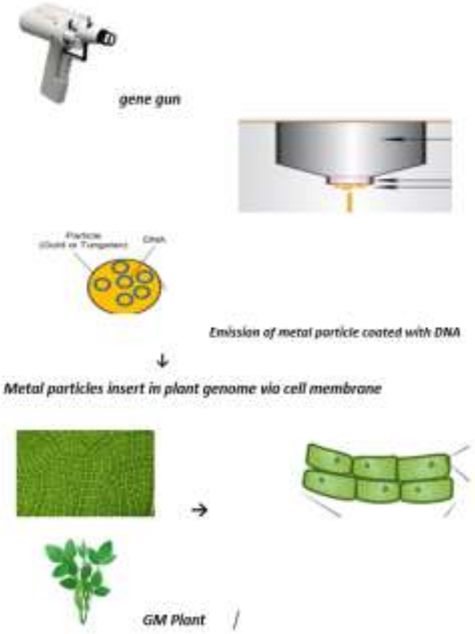

Diagram illustrating gene gun method for plants' genetic modification

*2.2.*Electroporation

Electroporation is employed when there is no cell wall present in the plant tissue. DNA enters plant cells briefly through microscopic holes created by electromagnetic pulses. For the creation of transgenic plants, electroporation is a well-known technique. Short bursts of high-voltage pulses can permeabilize the plasmid cell membrane. This makes it possible for plasmid DNA to be ingested and then transcribed reversibly before being permanently incorporated into the genome(Napotnik and Miklavčič 2018, Ozyigit 2020). In general, GM crops have one to three copies of the foreign gene, and all clones are typically integrated into a single region of the genome. Recombination and ligation processes commonly modify the plasmid DNA that has been introduced, and the number of copies does not necessarily correspond with the degree of alteration. Although the cell membrane is commonly thought to be impenetrable to DNA, electroporation can cause several undamaged cells and tissues to take up DNA (Ozyigit 2020).

*2.3.*Microinjection

We immediately put the foreign DNA into the cell using microinjection. According to plant experts, the outcomes of contemporary complete crop composition profiling demonstrate that crops modified utilising GM methods are less likely than traditionally grown crops to experience unanticipated alterations. The study's most frequently altered plants appear to be tobacco and Arabidopsis thaliana because of their easy propagation, well-studied genomes, and well-developed transformation technologies(Sharma, Bhatnagar-Mathur et al. 2005).They serve as model organisms for other plant species. For the introduction of new genes into plants, a promoter specific to the site of gene synthesis is necessary. For instance, a gene is only produced in rice grains as opposed to leaves when an endosperm-specific promoter is used (Low, Yang et al. 2018).

3. GM Traits

GM crops have been genetically modified to have a variety of traits, both in those that are now on the market and those that are being researched(Dizon, Costa et al. 2016). Some of these traits include a longer lifespan, tolerance to disease, herbicides, and insects, the capacity to create beneficial products like biofuel or medications, and the capacity to absorb poisons. GM tomatoes with a long shelf life were the first genetically modified plant authorised for sale in the US(Zimny, Sowa et al. 2019). The ability of Arctic Apples to develop resistance to the medicine kanamycin is caused by the presence of a gene for bacterial antibiotic resistance. In a research utilising tobacco plants, yields in terms of the weight of dry leaves produced were 14–20% higher(Craig, Tepfer et al. 2008).

Some GM plants, such as soya beans, provide the oil profile create to alter the plant's ability to collect a high quantity of oil that is comparable to fish oil(Dizon, Costa et al. 2016). Golden rice created by IRRI in 2016 yields a significant quantity of vitamin D, although it hasn't been grown in any nations since. The USDA certified a potato species in November 2014 that inhibits importation and produces less acrylamide. The first GM crop to be authorised for sale in the US was Monsanto's maize(Eckerstorfer, Engelhard et al. 2019). In salt-tolerant crops, a number of mechanisms for salinity tolerance have been discovered. Several crops, including rice, corn, and tobacco, have been genetically modified to express Bacillus thuringiensis (Bt) insecticidal protein genes(Rocha-Munive, Soberón et al. 2018). The cucumber mosaic virus has been designed to withstand viral diseases in papaya, potatoes, and squash. Squash was the second GM crop approved by the FDA in the United States(Eckerstorfer, Engelhard et al. 2019).

4. Risk

Risk is pervasive and cannot be eliminated. The likelihood of harm is a common way to describe risk. The prospect of (financial) loss or bodily danger is denoted by the phrase "risk"(Conner, Glare et al. 2003) The likelihood, possibility, and probability of an event are what have a negative influence. The management of probability may have an impact on the risk(Craig, Tepfer et al. 2008).

Knowledge and comprehension of damage and consequences are essential for risk interpretation. Different methods are used to quantify the appropriate risk factors, and this is usually done unintentionally(Rajan and Letourneau 2012). In GM crop risk problems, the ecology and toxicity of GM crop release and usage are investigated. Countries and stakeholders disagree about whether socioeconomic factors including sustainability, globalisation, ethics, and ethics should be taken into account when evaluating the danger of GM crops(Mueller 2019).

5. Risk assessment:

Enhancing quality, whether it is the quality of products or the quality of life, requires careful consideration of risks. An essential step in risk assessment is the identification of circumstances that might have negative effects (risk identification; what is not advantageous)(Prakash, Verma et al. 2011). An RA is an ongoing process that involves multiple assessment processes before offering findings with a tolerable degree of uncertainty. It should be understood that 100% certainty or zero risk can never be reached in a safety evaluation. Social, economic, and political factors may have an impact on the tolerable amount of uncertainty(Craig, Tepfer et al. 2008). GMO risk evaluation should be carried out via scientific procedures. There are now many different GM organisms being created, each with unique recombinant genes and characteristics. Risk assessment is today extremely intricate and imprecise, requiring the development of both highly specialized criteria for various organisms and features(Jouzani 2012).

Strategies to lessen or eliminate the dangers associated with GM plants are created when the risks are identified. The release of transgenic agricultural plants carries two different kinds of risks: The first is unintended consequences on the target population, such as when a transgene in GM crops or plants spreads resistance to consumers, a pest or disease negatively affects a person's health, and a pathogen or pest develops a specific resistance(Keese, Robold et al. 2014). Second, changes in biodiversity occur in non-target populations as a result of dangers brought on directly or indirectly by the introduction of GM crops. Risk assessment is aided by knowledge and experience related to GMOs. RA is a procedure that is only used in specific circumstances(Conner, Glare et al. 2003). For the specific type of organism, the information needed for evaluation must be stated; there may be information constraints. These details include the kind and properties of the gene product to be altered, as well as how the inserted gene(s) are expressed in the GM plants(Craig, Tepfer et al. 2008). Risk evaluation takes into account the crop's composition, nutritional value, potential toxicity, and allergenicity of gene products. Investigations are also conducted into how processing affects the qualities of crops and food(Conner, Glare et al. 2003). During the transformation processes, genetic engineering technology may have certain particularly unanticipated impacts, such as an arbitrary number of inserted copies in transgenes and changes to the inserted location and genetic material(Keese, Robold et al. 2014). Therefore, examination at each stage of gene creation and expression is essential. The most important thing is the assurance of stability and its inheritance for future generations.

International organizations such as the Cartagena Protocol on Biosafety, the Convention on Biological Diversity (CBD), and the International Plant Protection Convention (IPPC) assess the environmental consequences of GMOs. They are responsible for the characterization and assessment of GM crops before releasing them into the environment(Prakash, Verma et al. 2011).

1. Statistical methods of analysis(Craig, Tepfer et al. 2008).
2. Takes into account the different components used to GMOs are created (such as the properties of the GMOs) the donor organism, the vector, and the DNA that was inserted),
3. Assesses the finished product as a whole (characteristics) facts about the organism with new features to the intended function and possible qualities atmosphere of reception)
4. Takes into account relevant data gathered from both sources governmental and private research institutes and from international organizations(Rajan and Letourneau 2012).(Turnbull, Lillemo et al. 2021)

6. Risk associated with GM crops:

*6.1.*Presence of antibiotic resistance gene in GM plants

Risk evaluations usually look into the impact of antibiotic resistance on the organisms that come into contact with GM crops(Bawa and Anilakumar 2013). These indicators raise the possibility of drug-resistant bacteria. It is recommended that the use of such markers in crops be assessed. There is a risk that ARGMs from GM plants may transfer to bacteria under unusual circumstances, and if this happened, the damage would be minimal(Batalha, Foroni et al. 2021). There is still no known mechanism for how bacteria become drug-resistant. ARMGs should not be employed in transgenic plants when specific antibiotics are the sole available therapy for a certain clinical disease.

*6.2.*GM proteins

There have been worries that proteins made from GM organisms might reduce the availability of amino acids required for typical plant chemical synthesis. Although transgene-encoded proteins typically only accumulate 5-25% of total soluble protein (TSP), they have the potential to accumulate up to 46%(Rocha-Munive, Soberón et al. 2018). Damage to normal plant production is possible. Additionally, adjustments to the quantity of one protein or another protein may have negative nutritional effects. The toxicity, allergenicity, pleiotropic effects, and horizontal gene transfer risks associated with genes that code for enzymes and their byproducts are negligible(Zhang, Wohlhueter et al. 2016). By removing pointless risk assessment, evaluating these principles early in the construction of a GM facility can assist to narrow the scope of the safety reassessment. Additionally, GM plants could be designed to reduce the potential for environmental risks by incorporating specific genetic features, such as the absence of selectable markers and sterility.

*6.3.*Interbreeding

GMOs may breed with their sexually compatible or wild-type ancestors. If the specific trait is not preserved in wild forms that are advantageous to the transgenic, it may be lost(Prakash, Verma et al. 2011). As a result, wild creatures may become more tolerant, which might have an impact on their social interactions and ecological interactions with other species in their ecosystem.

7. Horizontal gene transfer

The non-sexual transmission of genetic material between members of the same species is referred to as horizontal gene transfer. It is the origin of potentially harmful elements like genes for antibiotic resistance. Recombinant DNA from genetically modified crops is found in soil locations, where it is ingested by naturally existing bacterial cells (rhizosphere) and integrates with their genomes rather than the intended site. This potential was found when transgenic sugar beet DNA was used to alter an ***Acinetobacter sp***. strain(Craig, Tepfer et al. 2008). Although there is no evidence of horizontal gene transfer (HGT), it is taken seriously when evaluating the safety of GM crops. In soil regions exposed to free rDNA or GM plant material, recombinant DNA has been found(Batalha, Foroni et al. 2021). Prior to reaching critical regions like the small intestine, large intestine, and other relevant portions for a certain amount of time, DNA may remain stable throughout the human gastrointestinal tract (HGT). Although some tests have shown that transgenes may spread to soil bacteria by horizontal gene transfer, there is no proof that this really happens(Bawa and Anilakumar 2013). In normal soil conditions, the selection pressure required for transfers from transgenic plants into the recipient bacteria, as investigated, would be extremely rare(Keese, Robold et al. 2014). Genes from a GMO may be spread by HGT to several species, including those that might become pests or ill. Because of horizontal transmission, these creatures' ecological niche or potential has changed(Prakash, Verma et al. 2011).

8. Risk assessment in Pakistan

To market GMOs and examine the product's impact on biodiversity and unintended species, risk assessment is crucial. Due to subpar performance, including disease susceptibility and the failure to deliver higher yield outcomes, PSC rejected 19 Bt-cotton cultivars. To enhance the risk assessment process, the Center for Environmental Risk Assessment (CERA) provided funding for over 20 projects for the South Asia Biosafety initiative.

9. Ways to manage Risks

The first evaluation may be validated with the help of managing risk and remedy. The dangers can vary depending on the type of GMO, how it must be implemented, and the ecology in which the GM plants are introduced. As a result, each one has to be assessed and managed separately(Rajan and Letourneau 2012). Each release is handled as a distinct entity since every GM plant has a unique collection of genetic features and genetic characteristics. It is wise to request government approval before conducting field trials and purposeful releases of genetically modified crops. This is especially true for genetically altered bacteria that can dwell in the ecosystem, survive there, and grow(Keese, Robold et al. 2014). The European Food Safety Authority recommends taking into account the following factors: (EFSA).

1) A mix of laboratory testing, experiments, and small-scale field exposures can be used to detect risks and estimate real-world exposure levels.

2) Extrapolating results from one setting to another, such as from research laboratories to limited field trials to industrial scale, is not appreciated(Rajan and Letourneau 2012).

3) Limited trials use fewer GMOs and can provide useful information on issues like survival and tenacity, compete efficiency, and some environmental impact of dispersal(Craig, Tepfer et al. 2008).

4) Advertisement dissemination, on the other hand, requires the release of a bigger number of GMOs in a variety of diverse ecosystems, and thus should be skilfully monitored regularly and at numerous sites to determine the impact on ecological relationships and ecosystem linkages.(Turnbull, Lillemo et al. 2021) In general, the risks connected with the use of genetically modified crops could be mitigated by risk-management strategies, making some planned behaviors acceptable. Confinement and monitoring are two ways to do this(Keese, Robold et al. 2014).

The environmental assessment of the GM plant in question will take into account the stewardship plan for genetically modified organisms (GMOs) post-release monitoring as a crucial component of a thorough full implementation for uncontrolled or marketed release. It is crucial to carry out this strategy(Turnbull, Lillemo et al. 2021). Future biosafety legislation may make monitoring and surveillance of GM crops important, however it might be challenging for research institutions, applicants, and auditors to discover reliable monitoring and surveillance solutions. Disease in GM crops must constantly be tracked and disease severity in crops after the release of any GM crop must be documented. The influence on soil organisms that prevent plant disease may be the cause of the unusually high disease incidence.

Questions have been raised. Weed, insect, and disease populations will be under a lot of stress from the introduction and widespread cultivation of GM crops(Keese, Robold et al. 2014). The agricultural and seed businesses are fully aware that this is not a brand-new issue in agriculture. A chronic problem in modern farming, crop management strategies are being developed to stop the emergence of disease resistance to current varieties(Rajan and Letourneau 2012). To lessen Bt resistance, "organised" zones might be implemented (an area of non-GM crop that is placed either within a crop or as a separate block within close proximity). New information and evidence, like as evaluations of the distribution of virulence factors and gene expression, may indicate that the size of an existing organised refuge is suitable be lowered in the future(Eckerstorfer, Engelhard et al. 2019). To reduce the development of resistance, stacked genes with comparable mechanisms of action, large doses, and a shared pest range will likely require smaller structured refuges(Turnbull, Lillemo et al. 2021).

10. Adverse Effects on the Health of People or the Environment.

These include enhanced pathogenicity, emergence of a new disease, pest or weed.

10.1. Undesirable effects:

When a transgene is delivered during transformation, it replaces the target component with a different transgene that has altered physical and functional characteristics. Similar results are produced by GM cotton's deformed cotton balls. There is more lignin in GM soy.

10.2. Toxicity:

The transgene's integration into a microbial vector can occasionally change the level of protein expression. Enzyme activity or inhibition may affect the capacity to create harmful compounds or cause antinutritive effects by binding certain nutrients and interfering with the metabolic pathway(Bawa and Anilakumar 2013). In a regular diet, a big portion of proteins are ingested without harm, but just a little portion can be harmful to health(Dizon, Costa et al. 2016). There are several plants that generate poisons and anti-nutrients, and GM cultivars could have larger concentrations of these materials. In a completely speculative scenario, it is possible that additional genes may be added or expressed to reactivate dormant manufacturing pathways.

It is believed that all proteins operate similarly to the proteins found in food and breakdown into amino acids. It may be exposed to whole proteins or large pieces if a protein has been shown to be resistant to typical digestive fluids(Prakash, Verma et al. 2011). This digestive resistance would produce a different analysis if the protein was broken down properly. The efficacy of the current toxicological technique to predict potential long-term health impacts of consuming genetically modified food is still under question(Dizon, Costa et al. 2016). Since cry proteins have a pesticidal (i.e. damaging) acute mode of action, toxicology studies were necessary. Short- and long-term toxicological testing have demonstrated that Bt proteins are safe for ingestion by both humans and animals, as is discussed in the following sections.

When proteins are ingested, they are not carcinogenic, genotoxic, or mutagenic. Given that Cry proteins are easily absorbed and can be harmed and rendered inactive during routine food preparation, ongoing testing for them is likely to significantly improve the stability analysis of GM crops(Koch, Ward et al. 2015). As long as food is consumed, proteins are broken down into amino acids and reassembled into new proteins, not accumulating in the environment. In acute toxicity tests, NOAELs for a range of recombinant proteins and Bt microorganisms were administered to mice at dosages 1000s to 1,000,000 times greater than those that rendered the target insects fatal(Koch, Ward et al. 2015). An adult would need to ingest roughly 900,000 kg of unprocessed Bt maize seed in a single day in order to acquire the equivalent acute dosage of Cry1Ab protein administered to mice without experiencing any undesirable side effects single day(Farias, Viana et al. 2015).

10.3. Allergenicity

A GM protein may cause previously undiagnosed allergies or mix with other airborne particles in sensitive individuals. Methionine from Brazil nuts can cause a number of immunological reactions, including allergic hypersensitivity. Although heat-stable proteins are more harmful, the allergenicity potential of a novel protein is minimal if it is heat extremely unstable and digestible(Dona and Arvanitoyannis 2009). A GM crop allergy investigation seeks to identify any possible allergic risks associated with a recently introduced protein. Bt bacteria are not thought to cause allergies in people since, despite being widely used as an insecticide for many years, there has only been one example of an allergic reaction documented(Dona and Arvanitoyannis 2009). The bacterial proteins in the Bt microbiological combination were also attributed for this specific allergic reaction, in addition to the Cry proteins(Bawa and Anilakumar 2013). Another approach to gauge how allergenic a protein is added is by how the body reacts to it. The amount of protein in the grain and the stability of the protein in the presence of pepsin are used to calculate exposure. There is evidence that certain allergens are present in food crops in large quantities and/or are resistant to digestion, although non-allergenic proteins share the same properties(Maghari and Ardekani 2011). The Cry proteins found in Bt crops have a limited ability to cause allergies when paired with other data, which indicates that they are not dangerous and have a low risk of causing allergies(Koch, Ward et al. 2015). No one model can meet the requirements for the ideal model, which would account for gastrointestinal and dermatologic sensitivity in addition to respiratory allergies. due to genetic differences. The ability to sensitise or alter gene expression may not be readily measurable across species(Rajan and Letourneau 2012).

10.4. ARG effects in humans

The effectiveness of antibiotic therapy may be decreased if resistance genes employed as transgenic agricultural markers are horizontally transferred to pathogenic gut bacteria. An example of a marker gene is the starfish fluorescent dye protein (GFP) gene(Dona and Arvanitoyannis 2009). GFP was proven to be harmless in the one study that precisely tested its toxicity and pathogenicity in male mice for four weeks.

The following elements are taken into account when assessing the safety of GM foods: the ability to trigger allergic responses (allergenicity) and the lack of major health impacts (toxicity)(Prakash, Verma et al. 2011). To guarantee the safety of transgenes and the absence of any potentially harmful impacts on nutrition and health due to genetic alteration. Identify any potential negative effects that the gene may have insertion(Batalha, Foroni et al. 2021).

11. New methodologies/approaches

11.1. Non transgenic molecular methods

To reduce the hazards associated with microbial vectors, scientists are advancing toward a method of transferring the transgene without introducing any microbial vector into recipient species. Some viral strains are being employed in genetic engineering to transfer genetic material in plant cells(Yin, Han et al. 2015, Zaidi and Mansoor 2017, Oh, Kim et al. 2021). They multiplied and survived without being integrated into the DNA of the host. Both DNA and RNA viruses (also known as tobacco rattling viruses) have demonstrated effectiveness against crops(Oh, Kim et al. 2021). Yellow bean dwarf virus, wheat dwarf virus, and cabbage leaf virus are a few examples of such DNA viruses. Viral vectors are used because of their effective machinery and genomic structure; they have shown to be excellent choices for vectors(Mahas, Ali et al. 2019).(Oh, Kim et al. 2021)

The open reading frames of Gemini viruses, which have a size of 2.8 kb, overlap one another. The fact that geminiviruses infect a variety of plant species, including wheat, cotton, tomato, maize, and beans(Yin, Han et al. 2015), makes them a good candidate for plant genetic engineering. Sometimes it also affects weeds and decorative plants. They are the perfect vectors for different hosts because of their ability to infect a wide range of host plant types. Recombinant replication, which is reliant and produces a high number of replicons, starts within the host cell and only needs one protein, Rep (replication-related protein; RepA). It is more effective since it enhances the capacity to target(Zaidi and Mansoor 2017, Oh, Kim et al. 2021). Their goal is for plants and crops to express heterologous proteins and their activities.

As infectious particles, viruses must be rendered non-infectious in order to be utilised as a vector. This can be accomplished by changing the genes, membrane coding sequences, and coat proteins of the virus(Yin, Han et al. 2015). Plant-to-plant transmission and cell-to-cell mobility are both eliminated as a result of this substitution. The plant genome is altered by modern genetic engineering to incorporate geminivirus resistance and improve immunity to other viruses(Oh, Kim et al. 2021). The pathogenic plant virus known as the tobacco rattle affects a variety of plant types. It has non-structural proteins and is a ssRNA positive virus. These structural proteins act as cloning sites for the integration of genes and other interesting elements during cloning(Zaidi and Mansoor 2017). Agrobacterium (least preferred) and another plant delivery method are used by TRV to alter plant species in particular(Oh, Kim et al. 2021). Because viral RNA genomes do not fuse with plant genetic material, small genome sizes are ideal for transformation purposes(Oh, Kim et al. 2021). The TRV-mediated transmission is supported by the CRISPR/Cas9 delivery system of contemporary technology, which also speeds up tissue culture procedures(Mahas, Ali et al. 2019, Oh, Kim et al. 2021). Transgenic plant growth and production are aided by the adoption of the Cas9 delivery system(Mahas, Ali et al. 2019). By removing the foreign DNA from GM plants, the alteration is accomplished(Zaidi and Mansoor 2017).

Limitations of employing Geminivirus as a vector to get around RNA virus restrictions(Oh, Kim et al. 2021). Such conditions are overcome by producing a DNA replicon that can act as a GT repair template(Yin, Han et al. 2015). Some disadvantages regarding viral vectors are that genetic material may lose during meiosis. New traits not expressed.

11.2. Genetic modification of microbes

Before the advent of modern biotechnology, microorganisms were genetically altered by exerting selection pressure or random mutagenesis caused by chemicals or UV irradiation(Eckerstorfer, Engelhard et al. 2019). Changing traits without a recognised genetic basis are the only ones for which this technique is currently relevant. Nevertheless, it has provided the framework for the development of a number of reliable, well-studied, and secure microbial production platforms for the expression of new traits via genetic engineering. When mutagenesis interacts with a plant's genome, it changes replication strategy, regulates the synthesis of toxins, and is triggered by chemicals and UV(Batalha, Foroni et al. 2021) The four main GE platforms are meganucleases, zinc finger nucleases, transcription activator-like effector nucleases, and clustered regularly interspaced short palindromic repeats/CRISPR associated9 (CRISPR/Cas9)(Hanlon and Sewalt 2021). The usage of sequence-specific nucleases (SSNs), often referred to as "designer nucleases," is a feature shared by all of these systems(Zaidi and Mansoor 2017)'(Batalha, Foroni et al. 2021).

11.3. Nano-carrier Technology: Better crops without genetic modification

Transgenic crops also pose questions regarding biosafety for people, animals, and the environment. Therefore, foliar spraying with high-throughput bioactive molecules may be a better method for crop improvement(Ahmar, Mahmood et al. 2021). Nano-carrier technology makes it possible to introduce biomolecules from the target genome into plant cells quickly and easily rather than using expensive and time-consuming biomolecule transfer processes(Thagun, Horii et al. 2022). (Abdurakhmonov, Ayubov et al. 2016). The benefit of this method is that the plant genome is not permanently altered or interfered with. Plant metabolic capacities may be effectively modified using this technology's small interfering RNAs (siRNAs) and double-stranded DNA fragments. The transport of molecules via nano-carriers may be advantageous for plant development. A variety of metallic, nonmetallic, and polymer-based nano-carriers have been connected to bioactive molecules(Wu and Li 2021). When sprayed, these biomolecules reach plant cells' cytoplasm directly. Because of the cellulose and polysaccharide network found in plant cell walls, which aids in translocation. But according to new research, foliar spraying also affects a plant's ionic channels in the leaves and stomata(Thagun, Horii et al. 2022). These biomolecules in plant cells have also been protected by nano-carriers from various degradation processes(Ahmar, Mahmood et al. 2021). These findings point to the potential for systematic quality attribute improvement in commercial crops using nano-carriers(Wu and Li 2021). The transmission of nano-carrier composites from specific plant organelles as well as across plant cell borders is facilitated by polypeptide networks found inside plant cells. Short amino acid sequences known as "cell-penetrating peptides" (CPPs) are employed in foliar spraying and have the ability to permeate plant cell walls and cell membranes. Cationic CPPs like KH9 and R9, for instance(Thagun, Horii et al. 2022)

Organelle-targeting peptides and chloroplast-targeting peptides are a couple of the peptides that aid in the transfer of nano-carriers from organelles(Thagun, Horii et al. 2022). These peptides' amino acids bind to specific substances in plant cells. CPP complexes with transduced gene expression in plant cells showed efficient DNA molecule translocation into the nucleus as well as momentary expression of external genes(Thagun, Horii et al. 2022). OTP-based nanocarriers were successful in delivering biomolecules into plant mitochondria and chloroplasts. Additionally, the extracellular plasmids on CPP's surface were modified to increase the transgenic expression of proteins in mitochondria and chloroplasts. This technology shows how biomolecule delivery techniques for plants have advanced(Ahmar, Mahmood et al. 2021). To deliver plasmid-encoding transgenic DNA to plant cell nuclei and other organelles, cationic CPP and OTP are combined with syringe infiltration, vacuum/compression infiltration, or injection. These approaches, however, cannot be used to reprogram plant quality traits in agricultural settings(Wu and Li 2021). The advantage of this method is that it is inexpensive.

Different CPPs shown variable degrees of translocation into plant cells following syringe infiltration. After spraying, fluorescein tetramethylrhodamine (TAMRA)-tagged cationic lysine/histidine (KH9 and R9) showed a higher penetration ratio into plant cells(Ahmar, Mahmood et al. 2021, Thagun, Horii et al. 2022). By modifying gene expression, it is possible to inhibit the metabolic processes carried out by mitochondria and plastids in plants. It may aid in the effortless distribution of plasmids to plant cells. The delivery system in plant mitochondria and plastids is not promoted by the RNAi mechanism, according to research(Ahmar, Mahmood et al. 2021). It has been established that chloroplasts house a significant number of small noncoding RNA molecules. It is not yet understood how they affect the post-transcriptional regulation of plastidial mRNA(Abdurakhmonov, Ayubov et al. 2016). This might be owing to a lack of a viable carrier for transporting a short RNA molecule introduced target organelle to examine its role in the RNA suppression mechanism.

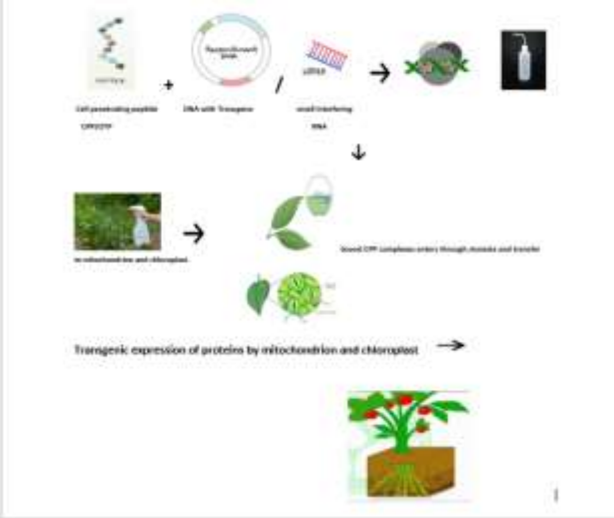

Diagram illustrating nano-carrier technology (foliar Spraying) Genetic modification

12. CRISPR

In bacteria and archaea, there is a complex adaptive immune system called the CRISPR system. In 1987, it was found for the first time in the Escherichia coli genome. CRISPR-Cas has been successfully developed into a powerful tool for editing the genomes of people, animals, and plants(Wang, Zhang et al. 2019). There are five different kinds and two classes of CRISPR/Cas systems. The guide RNA (gRNA or sgRNA) and the Cas9 protein are the two main elements of the system. Numerous genome-editing capabilities, including gene knock-in, knock-out, knockdown, and expression activation, have been demonstrated(El-Mounadi, Morales-Floriano et al. 2020).

SSNs cause double stranded breaks (DSBs) at or close to the target site by targeting a particular nucleic acid sequence. SSNs offer significant time and cost savings compared to conventional plant breeding techniques.Through the introduction of synthetic single guide RNAs intended to lead Cas9-mediated cleavage at selected locations(Zaidi, Mahas et al. 2020).

Guide RNAs are designed to precisely point Cas9 at the desired gene. Additionally, dynamic expression vectors enabling the cloning and simultaneous expression of Cas9 and guide RNAs have been created. Plants are modified utilising colonies expressing the CRISPR-Cas9 construct through a process called agrobacterium-mediated transformation(El-Mounadi, Morales-Floriano et al. 2020). To identify first-generation transgenic plants, herbicide or antibiotic selection is applied. The presence of the CRISPR-Cas9 cassette transforms the plants into transgenic organisms, subjecting them to biosafety regulations. Genome editing using CRISPR/Cas9 has been described in 41 food crop species, 15 industrial crop species, 6 oil crop species, 8 decorative crop species, 1 fibre crop species, and a feed crop species(Wang, Zhang et al. 2019). Through metabolic engineering and host gene control, this approach is still employed to improve crops.CRISPR-Cas9 has the potential to induce precise, site-specific genome editing.

13. Pros and cons of GM crops

*13.1.*Pros:

To provide foods desirable characteristics, manufacturers use genetic manipulation.Some of the potential advantages of GMO crops include:

- Apples and potatoes with a lower risk of bursting or turning brown have a higher market appeal.
- Improved flavour prolonged life span, leading in less waste and improved virus and other illness resistance(Dizon, Costa et al. 2016).
- Increased herbicide tolerance could potentially reduce waste and improve food security.
- Increased nutritious value, like that present in golden rice, that can assist people with limited food access improve their health(Rocha-Munive, Soberón et al. 2018)
- Higher insect resistance, allowing landowners to use herbicides and pesticides; ability to thrive in harsh circumstances, such as drought or heat; and the ability to grow in saline soil
- Increasing the resistance of plants against pasticides(Koch, Ward et al. 2015)

*13.2.*Cancer

There has been concern that eating GMO foods may make cancer more likely by raising the body's concentrations of chemicals that may cause cancer. The American Cancer Society claims that there is no proof that eating GMO foods increases or lowers cancer risk. There is no evidence to show that changes in cancer rates over time in the United States are related to the introduction of genetically modified foods(Prakash, Verma et al. 2011). It could take several years for a trend to appear even if a relationship is found.

*13.3.*Antibacterial resistance

A few GMOs have undergone modifications to develop antibiotic resistance. These plants' genes may theoretically enter the bodies of people or animals who consume them(Dizon, Costa et al. 2016). The behaviours of humans or animals may lead to the development of antibiotic resistance. Although the likelihood of this occurring is remote, the WHO and other health organisations have established regulations to guard against it.o

*13.4.*Change in human DNA

Some food experts believe that food DNA can survive in the stomach, which has led to worries about possible immune system harm. Additionally, some people worry that consuming GMO foods will alter our genetic makeup(Maghari and Ardekani 2011). However, before food reaches the large intestine, most DNA in it, whether it is from GMOs or not, is eliminated or broken down. There is no evidence that food-borne DNA fragments have any impact on human health or genetic composition, despite the fact that they can penetrate the circulation and internal organs(Prakash, Verma et al. 2011).

*13.5.*Toxicity for body organs

According to certain 2009 research, eating GMO foods may have an impact on the reproductive system, pancreas, liver, and kidneys. Due to a lack of supporting data, they were unable to confirm this and asked for more study. Applying GMO crops may also reduce the chance of chemical poisoning as farm owners may avoid using pesticides that have proved dangerous in the past(Bawa and Anilakumar 2013).

14. GM crops status in Pakistan

The two most significant GM crops in Pakistan are cotton and maize, both of which are resistant to weeds and insects. In Pakistan, bt cotton was initially planted as a genetically modified crop in 2002(Kouser, Spielman et al. 2019). Four cultivars of Bt-cotton with insect resistance (IR) were commercialised by the Pakistan Atomic Energy Commission (PAEC) in 2005, which led to less pesticide use and increased agricultural output(Babar, Nawaz et al. 2020). 50 more Bt-cotton cultivars were approved for commercialization between 2013 and 2016 by the National Biosafety Committee (NBC), PSC, and Pakistan Central Cotton Committee (PCCC)(Kouser, Spielman et al. 2019). With 3 million hectares planted, Bt cotton currently produces 96% of all cotton produced in Pakistan.

Additionally, Pakistan's maize crop is dealing with insect resistance and weed stress. The introduction and marketing of various GM/Biotech herbicide-tolerant (HT) and insect-resistant (IR) maize varieties are believed to allay these worries(Babar, Nawaz et al. 2020). hese biotech cultivars were approved by the PSC and NBC in 2016, and production started in the provinces of Punjab and KPK in early 2017. Over the following ten years, farmers are anticipated to make a net benefit of $1 billion (USD) through the use of these IR/HT maize varieties. Having produced GM cotton for more than ten years, Pakistani farmers are among the top producers of GMOs worldwide. It is clear that GM crops play a significant role in ensuring food security.Furthermore, GM crops are a cure for malnutrition and famine. Research on various crops is also being conducted in order to fight the primary concerns of global warming and climate change(Babar, Nawaz et al. 2020).

15. Need for GM crops

In 2012, 868 million people worldwide—more than two-thirds of whom lived in Asia and the Pacific—suffered from hunger and malnutrition, according to the United Nations

Food and Agricultural Organization (FAO)(Oliver 2014). Agriculture production would have to treble by 2050 in order to feed the world's expanding population. This is a challenging issue with several socioeconomic and political effects(Zhang, Wohlhueter et al. 2016). The UN Food and Agriculture Organization (FAO) states that by 2050, global food production must increase by at least 50%. This would need an increase in maize, wheat, rice, and soybean yields of 2.4 percent or 1.6 percent every year(Azadi, Ghanian et al. 2015). By 2050, agricultural yields will have do By 2050, each person on earth would have around 0.18 hectares of arable land available for food production, down from the current level of 0.242 hectares, according to an FAO estimate(Zimny, Sowa et al. 2019).

The production of biofuel feedstock requires additional land, and this does not take into consideration possible changes in land use brought on by urbanisation, desertification, salinization, and soil degradation. In the late 1950s and early 1960s, there was widespread hunger over much of Asia(Zhang, Wohlhueter et al. 2016). Modern breeding efforts are starting to be influenced by molecular and genomics-driven technologies, such as marker-aided breeding and genotyping by sequencing. This is the point at which genetically modified organisms (GMOs) and biotechnology start to take off(Oliver 2014). This is where biotechnology and the creation of Genetically Modified Organisms (GMOs) emerge(Hanlon and Sewalt 2021).

16. Challenges:

Genetically modified organisms (GMOs) should not be released into the environment or sold without first receiving approval from a risk management plan and scientific risk assessment(Bawa and Anilakumar 2013). The majority of the time, this is accomplished by taking into account all of the risks connected to a brand-new GM crop or derived product. By examining "risk hypotheses," or hypotheses that predict the likelihood of adverse effects, environmental risk assessments can offer high confidence of low risk(Turnbull, Lillemo et al. 2021). A broad and specific set of criteria for various GM species and features are required since risk assessment for genetically modified organisms (GMOs) is difficult and unclear. There are no international regulations governing the use of live vaccinations for animal usage, long-term storage of GM microorganisms, or the use of genetically modified microorganisms to manage pests and diseases(Turnbull, Lillemo et al. 2021).

Conclusions

It will need sound research and excellent communication to resolve the complicated dispute over assuring the safety of GM crops. There are currently no comprehensive recommendations or specific protocols for determining the safety of foods made from genetically modified (GM) crops, just generic evaluation criteria. While it is crucial to consider every possibility in order to prove that genetically modified crops are safe, previous studies shouldn't be ignored. Genetically modified crops have the potential to cure many of the world's hunger and malnutrition problems, and their enhanced production may also help to protect and sustain the environment. Although transgenic crops have a number of advantages and could provide answers to a number of problems, there must first be shown that these foods won't cause any new problems. These might be caused by a number of things, such as inserted genes and the proteins they create, pleiotropic or side effects of the results of gene expression, and perhaps disrupted endogenous genes in the transformed organism. A biosafety code that regulates the trans-border transit of genetically modified organisms has currently been established in several countries in order to mitigate possible risks to biodiversity, human health, and the environment at large. The development of gene sets and methods that serve as biomarkers for a cell's sensitivities to pesticides, allergens, or other chemicals is the promise of molecular genetics, toxicology, biochemistry, and nutrition breakthroughs.

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Conflict of Interest

The authors declare no conflict of interest.

Authors Contribution

SR conceived the original idea and designed the outlines of the study. All the authors equally contributed and wrote the 1st draft of the manuscript. SR revised the whole manuscript and formatted it accordingly. All authors have read and approved the final manuscript.

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