International Journal of Research in Engineering, Science and Management
Volume 5, Issue 3, March 2022
https://www.ijresm.com | ISSN (Online): 2581-5792

Understanding the Ethical and Environmental Implications of Genetically Modified Crops through the Bt Insecticidal Gene

Siwat Pongpaiboon*
Neerja Modi School, Jaipur, India

***Abstract*: Genetic modification has been used to alter the genetic makeup of organisms by adding or removing genes since 1973. The technology has great potential in the field of agriculture and food and it continues to evolve. Among many of the gene introduced into plants, Bacillus thuringiensis (Bt) gene was introduced into crops, primarily, maize, cotton, soybeans and rice. The gene makes the plant produce Cry proteins that are poisonous to targeted insects. Despite its scientific and agricultural promise, many concerns about the safety of human, animal consumption, agricultural land and the environment have been raised. These issues are caused by uncontrolled gene flows. Besides this, many countries also have to deal with the ethical concerns raised by the public, making the use of GMO controversial. his paper takes a total view of these concerns and attempts to review the potential risks of the Bt gene on aspects of agriculture, biodiversity and, human and animal health Upon evaluating existing literature, this paper argues that there is not enough evidence to conclude that the Bt gene can adversely affect human or non-targeted species.**

***Keywords*: bt gene, cry protein, GM crops, insecticidal gene.**

1. Introduction

Genetically modifying an organism involves inserting desired genes, or removing unwanted genes to alter the genetic makeup of an organism in order to get desired phenotype. To genetically modify an organism, the scientists identify its desired or undesired phenotype so that the corresponding gene can be inserted, replaced or removed. The specific gene can be found by Polymerase Chain Reaction (PCR) which replicates the genetic material so that it can be easily identified. After the gene is identified, the DNA needs to be extracted from the cell, by cutting the cell open and storing DNA. The separation of the gene is done by the process of gene isolation in which the DNA is broken using restriction enzymes cutting its restriction site. The section of gene is then inserted into the plasmid of a vector using ligase enzymes which makes a recombinant DNA. This recombinant DNA can be inserted into the genome of the cell or can exist as extrachromosomal DNA. The insertion can be done in many ways including,

1. *Transformation:* First demonstrated by Federick Griffith in 1928, it is a natural process where bacteria take up DNA from the environment, in cases where the bacteria are competent. In some circumstances, the host needs to be made competent by either calcium chloride induced transformation electroporation or protoplast transformation/fusion.
2. *Transfection:* This process artificially introduces DNA or RNA into the eukaryotic cells. The DNA introduced can be expressed on extrachromosomal plasmid or in the cellular genome. This method usually involves mixing nucleic acid with reagent containing positive charge to neutralize negative charge on DNA or RNA backbone.
3. *Transduction:* This process transfers genetic material from one bacterium to the other with the help of phages. The Bacteriophage has a tendency to attack the eukaryotic cell to deliver the DNA inside the host and replicate.
4. *Conjugation:* A process of transferring through a mating bridge during which, a bacteria acts as a donor and the other is a recipient. The donor then produces a pilus, drawing two bacteria together and transferring the genetic material across.

These methods are being used on prokaryotic and eukaryotic cells. The method commonly used to genetically modify a plant is by Agrobacterium tumefaciens method or particle gun method in which it is bombarded with metal coated by DNA of the desired gene.

The introduction of Flavr Savr tomato in 1994 helped in dealing with the rotting problems. The tomato was genetically modified to slow the ripening process, thus, increasing its shelf-life (Shukla et al., 2018). Thereafter, these crops started gaining popularity considering that with the world population expected to reach 10 billion in the year 2057, the demand for food will inevitably rise. Genetically modifying crops can increase the yield for food by lessening the maturation time. Moreover, making the crops resistant to weather conditions, pesticides or herbicides also contributes immensely towards sustainable development (Singh & Singh, 2017,296–316). This technology also has a potential to boost farmers' profit by 68%, increase the yield by 22% and, reduce the use of chemicals by 37%. Furthermore, these types of crops can also increase the nutrient values which can contribute towards the resolution of problems

---

*Corresponding author: siwat.p@nmsindia.org

such as malnutrition and various diseases like cancer and diabetes in developing countries (Smyth, 2020, 887-888). Table 1 shows examples of some of the registered GM crops. Despite its benefits, the Genetic Engineering Appraisal Committee of the Ministry of Environment, Forest and Climate Change of India has only approved Bt cotton while the rest of the other GM crops were banned with the concern of environmental health and biodiversity (The Economic Times, 2021). Many people disagree with this ban, thinking that it would increase their yield and income, in the same way that Bt cotton made India one of the largest cotton exporters in the world (The Hindu Business Line, 2021). According to DBT, the Government. of India, the country is still pursuing research on the commercialization of GM crops in seven incubation centers and Biotechnology parks.

Similar to India, the consequences of GM crops are being evaluated. The technology is suspected to affect non-targeted species by reducing biodiversity by pollinating and contaminating the natural gene (Bailleul et al., 2016). This would introduce the pest and herbicide resistance gene in the native species, causing these superweeds and superbugs to disrupt the food chain. Furthermore, it can also lead to soil toxicity. Some countries have difficulties in approving GMOs not because it would negatively impact biodiversity and human life, but also because it may encounter psychological, emotional and political opposition.

The field of genetically modifying crops is constantly developing. Plants are constantly being genetically modified with different characteristics. Recently in 2021, the University of California, Riverside noticed that 80% of the crops lost from microbial infections were caused by fungus (Ober, 2021). This initiated the development of natural antibiotics taking advantage of the plant's defense system. Fungi produces enzyme polygalacturonases to break down a plant's cell wall, and the plant produces the Polygalacturonase inhibitor protein (PGIP) to slow down the breakdown of the cell wall. The researchers located the DNA for production of PGIPs in common green beans and inserted the gene into yeast. After the yeast replicated the DNA, they were introduced into the culture of Botrytis cinerea and Aspergillus niger. The result was successful and has potential for large scale production of PGIPs. The protein produced is also biodegradable into natural amino acids, which will result in minimal impact on the environment. The tools in genetically modifying crops are also constantly expanding. Regenerate Genetic Algorithm technology is used to accelerate the breeding process in many crops. However, in grain legumes there is not yet any robust protocol. Without this technology, a crop cultivar depends on the number of years needed for developing homozygous lines from hybridization which could take many years. The findings from the attempt to produce seven generations per year enabling speed breeding in chickpea demonstrates a positive response on the earliest stage of germination (Samineni et al., 2020). This indicates the possibilities of the method working on a wider range of chickpeas. Another technology that revolutionized genetic engineering is CRISPR. It initially is an immune response of bacteria against viruses. They recognize and destroy DNA. Scientists use this property to accurately cut genome sequence in plants and fruit crops. From 2014 to 2018, this technology has been used to genetically modify many plants, including tomato, citrus, cucumber, apple, grapes, watermelon, kiwi fruit, etc., (Wang et al., 2019). The recent work in genetically modifying plants has been used in production of therapeutic proteins creating the plant derived therapeutic protein for human use in 2012. In 2019, plant produced influenza virus vaccine completed phase 3 clinical trials (Ward et al., 2020). Furthermore, a similar type of vaccine is being developed for SARs-CoV-2. It is expected that plant-made vaccines for influenza and SARs-CoV-2 will be the first therapeutic protein produced in whole plants for human use (Fausther-Bovendo & Kobinger, 2021).

2. Genetic Contamination

Maize is genetically modified to be resistant to pests such as lepidopteran larvae that are stalk borers (Svobodová et al., 2017). This is done by introducing the Cry gene of Bacillus thuringiensis or Bt which is toxic for the pests. The most serious ones among these are corn rootworms. The first Bt maize introduced in the US was a single trait Cry3Bb1 in 2003 (U.S. Environmental Protection Agency, 2010). Following this, Cry34Ab1/Cry35Ab1 was registered in 2005 as a single trait and Cry3Bb1 with Cry34/35Ab1 was registered in 2004 and mCry3A with Cry34/35Ab in 2012 (United States Environmental Protection Agency, 2015). The gene has the potential to flow from transgenic crops to its wild relatives producing new gene combinations to the population (Dively et al., 2019). The uncontrolled gene flow causes gene contamination. This often occurs when GM plants are within the range that they can cross pollinate with other plants. An example of hybrid species is creeping bentgrass that is genetically engineered to be pest resistant. The plant is wind pollinated, as a consequence, the research detects the pest tolerance gene in wild grass within the range of 9 miles one year after the grass was planted (Landry, 2015). As for maize, it is approximated that 97 percent outcrossing between plants can occur up to 200m. This can be influenced by environmental factors such as wind and temperatures (Dively et al., 2019). The gene flow from GM to non-GM crop is shown in Figure 1.

The uncontrolled gene flow can bring many environmental and pest resistance concerns which are unwanted by farmers. Moreover, the consequence of GM gene contamination is difficult to specify (Gudeta, 2019). Therefore, there are methods implemented to reduce genetic contamination. Seed blend refuge system is one of the methods used to slow the evolution of western corn rootworm. The method uses non-Bt corn planted adjacent to the Bt corn in order to avoid the pest's exposure to Bt toxin. The method is especially effective when the crop is a pyramid with Bt toxin targeting the same insect. As a result, the method reduces the Bt resistant offspring (Hughson & Spencer, 2015). This method, however, has certain limitations because of the gene flow. In 3 years of study, comparing the emergence of corn earworm in the seed blend refuge, the percentage of Bt kernels to nonBt had expression of bt toxin up to 76% (Vyavhare et al., 2021). The gene flow from

Bt to nonbBt refuge plant could reduce the effectiveness of this method as the pest is now more likely to come in contact with the Bt gene and becomes resistant (Yang et al.,2017), (Dively et al., 2019). Moreover, results from modeling pollen contamination in refuge-in-bag suggests that it increases the resistance for ear-feeding insects (Yang et al., 2017). For these reasons, insect resistant management, IRM does not consider this method to be appropriate for non-pyramid gene Bt maize. The study shows that uncontrolled gene flow can influence the methods used to control Bt and Cry protein resistance. Additionally, the gene flow can cause transgene escape that was found in many crops including maize to non-transgenic maize and landraces. The medium which is used in these gene escapes are all pollen (Rizwan M. et al., 2019). Genetic contamination then, can be a problem that is hard to solve for farmers and once the gene escapes into the environment it can disrupt the food chain. This causes unwanted ecological changes (Gudeta, 2019).

Table 1

Crop List	Name and Code	Developer	Method of Trait Introduction	GM Trait	Gene Introduced	Gene Source
Alfalfa (Medicago sativa)	Name:J101	Monsanto Company and Forage Genetics International	Agrobacterium tumefaciens-mediated plant transformation	Glyphosate herbicide tolerance	cp4 epsps (aroA:CP4)	Agrobacterium tumefaciens strain CP4
	Code: MON-00101-8					
	Name: KK179	Monsanto Company and Forage Genetics International	Agrobacterium tumefaciens-mediated plant transformation	Antibiotic resistance	ccomt (inverted repeat)	dsRNA that suppresses endogenous S-adenosyl-L-methionine: trans-caffeoyl CoA 3-O-methyltransferase (CCOMT gene) RNA transcript levels via the RNA interference (RNAi) pathway
	Code:MON-00179-5			Altered lignin production	nptII	neomycin phosphotransferase II enzyme
	Name: KK179 x J101	Monsanto Company (including fully and partly owned companies)	Conventional breeding - cross hybridization and selection involving transgenic donor(s)	Glyphosate herbicide tolerance	cp4 epsps (aroA:CP4)	Agrobacterium tumefaciens strain CP4
	Code: MON-00179-5 x MON-00101-8			Antibiotic resistance	ccomt (inverted repeat)	Medicago sativa (alfalfa)
				Altered lignin production	nptII	Escherichia coli Tn5 transposon
Apple (Malus x Domestica)	Name: GD743	Okanagan Specialty Fruits Incorporated	Agrobacterium tumefaciens-mediated plant transformation	Antibiotic resistance	PGAS PPO suppression gene	Malus domestica
	Code: OKA-NB001-8			Non-Browning	nptII	Escherichia coli Tn5t ransposon
Maize (Zea mays L.)	Name: 32138	DuPont (Pioneer Hi-Bred International Inc.)	Agrobacterium tumefaciens-mediated plant transformation	Male sterility	ms45	Zea mays
	Code: DP-32138-1			Fertility restoration	zm-aa1	Zea mays
				Visual marker	dsRed2	Discosoma sp.
	Name: 59122	Dow AgroSciences LLC and DuPont (Pioneer Hi-Bred International Inc.)		Glufosinate herbicide tolerance	pat	Streptomyces viridochromogenes
	Code: DAS-59122-7			Coleopteran insect resistance	Cry34Ab1	Bacillus thuringiensis strain PS149B1
					Cry35Ab1	Bacillus thuringiensis strain PS149B1
Tomato (Lycopersicon esculentum)	Name: B	Zeneca Plant Science and Petoseed Company	Agrobacterium tumefaciens-mediated plant transformation		pg (sense or antisense)	Lycopersicon esculentum
	Code: SYN-0000B-6				nptII	Escherichia coli Tn5 transposon
	Name: FLAVR SAVR™	Monsanto Company (including fully and partly owned companies)	Agrobacterium tumefaciens-mediated plant transformation	Antibiotic resistance	pg (sense or antisense)	Lycopersicon esculentum
	Code: CGN-89564-2			Delayed fruit softening	nptII	Escherichia coli Tn5 transposon
	Name: Da	Zeneca Plant Science and Petoseed Company	Agrobacterium tumefaciens-mediated plant transformation	Antibiotic resistance	pg (sense or antisense)	Lycopersicon esculentum
	Code: SYN-000DA-9			Delayed fruit softening	nptII	Escherichia coli Tn5 transposon

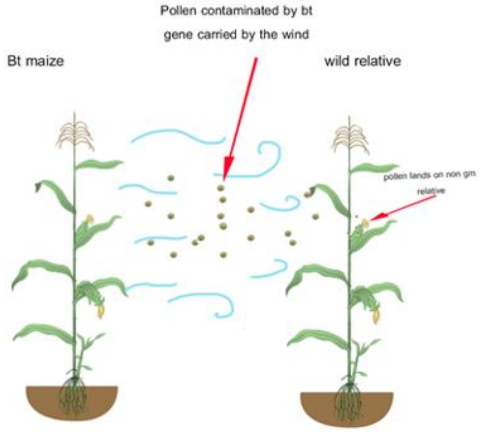

Fig. 1. Gene flow from GM Maize to non-GM Maize

3. Ecological Effects

The ecological effects of Bt crops are seen to be quite controversial. The use of herbicide tolerance crop systems on biological control can be difficult to predict. Once the transgene enters the ecosystem, the cross-compatible relative becomes more abundant from the absence of herbivore pressure. The increase of resistance against a certain species of insect then can change the balance of the food chain (Gudeta, 2019).

In order to investigate the toxicity of insecticidal protein, a risk assessment approach similar to that of pesticide is being used. It takes into account the species that are most likely to be sensitive (Romeis et al., 2019). Concentration of Bt protein can vary across different crops and across developmental stages (Romeis et al., 2019). Bt protein concentration becomes lower at the end of the growing season but this is not observed in Cry1Ac in maize. There are also problems associated with Cry protein exposure in pollen feeding organisms which was observed in predatory bugs when the prey is scarce (Meissle et al., 2014). Maize which shed large amounts of pollen can increase Bt protein consumptions. Some insects, like green lacewings, feed directly from the pollen in the adult stage. Furthermore, the pollen can be ingested passively when spiders clean their webs (Peterson et al., 2016). Soil inhabiting enemies might feed on the leaves or roots exposing them to Cry protein. Although the nectar of the plant might contain Cry protein, there has been evidence for a parasitoid of plant hoppers containing Cry protein when caged with Bt rice plants (Tian et al., 2017). Concentration of Bt protein depends on the part insects feed on. Aphids which feed on phloem sap are exposed to lower levels of Bt protein (Tian et al., 2015). On the other hand, herbivores that feed directly on green plant tissues for example caterpillars and spider mites consume high amounts of Bt protein (Meissle & Romeis, 2017). Spider mites are found to have the Bt protein concentration because it feeds on mesophyll cells where Bt protein is maximum. Parasitoids have similar exposure to that of the predators, as species that consume the gut of the host are exposed to more Bt protein while species that feed on nectars do not ingest significant amounts (Li et al., 2017). The exposure of Cry protein in insects can through direct or indirect consumption. Bt protein by some arthropods becomes undetectable within a few days of switching to non Bt one, suggesting that it can be digested or excreted out (Zhao et al., 2016). In cotton bollworms, however, there was Cry1Ac found in the body (Zhao et al., 2016). It is reported that the protein can accumulate in a ladybird (Paula & Andow, 2019). Overall, from ELISA measurement, the concentration of Bt protein in the food chain depends on the part that is consumed and can be passed on to their natural enemy. The concentration of Bt protein can also be diluted by digestion and excretion of animals.

However, with the current evidence, Bt and insecticidal proteins in GM crops such as Cry 1 and 2 cause no harmful on other organisms apart from Lepidoptera. Similarly, Cry3 proteins do not harm other organisms apart from its target pest (Coleoptera and Chrysomelidae). The proteins and target pest are shown in Table 2. Even Bt plants that produce two or more

Table 2
Insecticidal Bt gene and effects on organism

Target Pest	Gene	Effect
Coleopteran insects	Cry34Ab1	Confers resistance to coleopteran insects particularly corn rootworm by selectively damaging their midgut lining
	Cry35Ab1	
	Cry3Bb1	
	Cry3A	Confers resistance to coleopteran insects by selectively damaging their midgut lining
Lepidopteran insect resistance	Cry1A	Confers resistance to lepidopteran insects by selectively damaging their midgut lining
	Cry1A.105	
	Cry1Ab	
	Cry1Ab-Ac	
	Cry1F	
	Cry1Ac	
	Cry1C	Confers resistance to lepidopteran insects, specifically Spodoptera
Multiple insects	eCry3.1Ab (synthetic form of Cry3A gene and Cry1Ab gene)	Confers resistance to coleopteran insects by selectively damaging their midgut lining
Hemipteran Insects	mCry51Aa2	Confers resistance to hemipteran insects Lygus hesperus and L. lineolaris by selectively damaging their midgut lining

different insecticidal proteins hold no adverse effect on non-target organisms (Romeis et al., 2019). Studies suggest that this also holds true for combination of Cry proteins and dsRNA (Khajuria et al., 2018). Instead of harming the ecosystem, the evidence supports that Bt genes lead to higher insect diversity than monoculture with use of synthetic insecticides (Romeis et al., 2019).

4. Risk on Humans

Genetically modified plants may also pose risks to humans (Papadopoulou et al., 2020). RNAi interference method can lead to off-target effects (Casacuberta et al., 2015). The off-target effects can be used in risk assessment of GM crops. A large part of the human diet consists of ncRNAs and RNAs, however, these are destroyed after ingestion by acidic pH and enzymes in the digestive system. It can, therefore, be considered that the amount ingested is negligible until the RNA is modified to become more stable (Papadopoulou et al., 2020).

Most common food allergies in humans occur in the first few years of life-span due to an immature digestive system (Santis et al., 2018). Humans are exposed to Cry protein from kernels of the plant the same way the animals do, hence animal models are used in humans to identify potential hazards. According to theFood and Agriculture Organisation (FAO), the source of the gene can be the source of food allergies in people. Allergenicity is also assessed by an entire assessment of the GM plant and its protein (Dunn et al., 2017). In GM crops, bioinformatics is used to find out the protein sequence known for human allergies to see whether it can be cross-reactive with known allergens (Goodman et al., 2016). In 20 years, only 1 case of protein was prevented from commercialization due to allergic concerns – this was Brazilian nut protein which was cross-reactive to patients allergic to Brazilian nuts (Goodman et al., 2016). Cry proteins are also consumed by humans. Studies show that the consumption of Cry proteins by humans holds no adverse effect as it can be digested by digestive systems (Carzoli et al., 2018). The protein consumption is then considered safe when the level is low relative to the body weight. Moreover, there hasn't been much substantial risk to humans (Carzoli et al., 2018). Since the introduction of GM crops in 1996, there isn't a recorded case of human risk (National Academies of Sciences, Engineering, and Medicine, Division on Earth and Life Studies, Board on Agriculture and Natural Resources, Committee on Genetically Engineered Crops: Past Experience and Future Prospects, 2016). The cases reported in the 1990s resulted in suspicions for "apparent allergic reactions" for Starlink corn-containing products whose serologic testing failed to show immunoglobulin binding to protein in patients in placebo-controlled, double blind and food-controlled challenges. The only evidence available to support this was WHO approved Starlink premarket safety testing of Cry9AB protein. From the testing, it was theorized that the protein was digested at a slow rate, resulting in the immune system reconviction, but no actual allergens found (Sutton et al., 2003).

The risk of Bt maize on humans, with the current studies and past incidence, is very likely to be minimal. Even though rat, mouse and animal models are accepted as surrogates for human allergies, there are no human randomized or controlled studies (Dunn et al., 2017). This creates limitations to generalizability of these studies.

5. Agricultural Problems

Bt can lead to pest resistance which makes it more difficult for future pest control. Advisory Commission on Agriculture Biotechnology recognises the resistance of target pests as causes of reduction in the value or benefit of the GM crops (Signorini et al., 2018). The Bt gene in pyramided plants have created pest control problems in recent years (Huang, 2020). Take the case of Bt maize for instance. Within six years of its introduction, the first case of Bt resistant pest was observed (Gassmann, 2021). Since then, the resistance has become widespread throughout the US corn belt (Clair et al., 2020). In 2012, there was the first damage report by Diatraea saccharalis on the Bt hybrid in Argentina. The damage from the pest included bored stems and tunnels, causing the stem to break thereby reducing the yield. In the US, one of the most damaging pests is the western corn rootworms which is responsible for most of the yield loss, accounting for between $1-2 billion (Wechsler & Smith, 2018). In 2009, a high level of damage caused by feeding was observed in maize producing CryBb1 (Gassmann et al., 2011). The bioassay of the specific event suggests that it is caused by CryBb1 resistant corn rootworm (Gassmann et al., 2011). Several years later, there continues to be feeding injury from other CryBb1 or Cry3A fields and this was identified to be caused by rootworms that are resistant to these proteins (Gassmann et al., 2014). In case of feeding damage in a field in 2012, the bioessay demonstrated it to be caused by resistant rootworm. Except this time, it was suggested that there was a cross resistance between CryBb1 and Cry3A and was extended to eCry3.1 (Jakka et al., 2016). The sample field witnessed a damage equivalent to 15 to 17 percent yield reduction (Tinsley et al., 2012). These damages caused from the resistant pest show that maize can yield reduction which can be the loss of the farmers. The resistance in pests continues to develop, and the cross resistance in the protein CryBb1, mCry3A and eCry3.1ab is possible due to the similarity in structure (Jakka et al., 2016). While the other proteins are structurally different, the mechanism of Bt resistance in western corn rootworms still needs more research to be understood (Jurat-Fuentes et al., 2021). Moreover, the resistance to Cry34/35Ab1 is being investigated in research and there is evidence for its resistance (Gassmann et al., 2019). These studies suggest that the pest resistance genes are constantly evolving and the new ones have a high probability of being discovered in the near future. Even though there are many management strategies used to reduce the evolution of resistant pests, the gene flow of Bt maize makes it difficult to control. Overall, GM crops are comparatively expensive and may have ethical concerns.

6. Ethical Issues

With many concerns about GM crops, there are constant arguments against the technology, which has raised many ethical concerns. The ethical concerns raised differ in different countries depending on public beliefs on technological influence as well as religious faiths. The percentage of area of GM crops grown in some countries of the world is shown in Table 2. Most of the countries also have taken into consideration the risk to public health, economy and environment. The difference in the level of concern towards the technology can be seen from initial opinion polls for GMOs labeling which varied across different countries (Shanahan et al., 2001). In Europe, the labelling was mandatory while North America did not require such labeling (Akin et al., 2019). In Poland, it is stated that "genetically modified varieties shall not be included in the nation catalogue" and "the seed of genetically modified varieties cannot be accepted on the market in the territory of the Republic of Poland ". This concern has arisen due to the Christian populations' issue with the technology, which consists of most of its population (Zetterberg & Björnberg, 2017). Whereas in Mexico, during 1995 to 2017, 141 applications were rejected either after risk assessment or due to lack of information from the total of 893 applications received by CIBIOGEM. This makes a total of 15.8 percent of rejection rate (Ruiz et al., 2018). In the US there were arguments for labeling food using genetically engineered ingredients which the scientist's consensus being that the ingredients are more risky than conventional ones. Even though there are regulations against introducing GM crops into different countries, the European Union has been criticized for its inconsistencies in scientific point of view. There are also concerns about negative consequences on small businesses (Genetically Modified Organisms: The Case for New Regulations, 2013). Moreover, the North-American legislation has also been criticized for its lack of attention to process related preference of consumers by being insufficiently transparent. It also fails in providing opportunities for public participation (Do, 2012). On the other hand, GM food can also be labelled negatively, which can reduce the consumer's autonomy. As a result, some of the consumers prefer non-GM food over GM. The plant developer can give names to gene editing that can create a positive attitude since the inaccuracy in nomenclature can make the consumer feel that the developers are dishonest and with their products and lessen their trust (Kuzama, 2018).

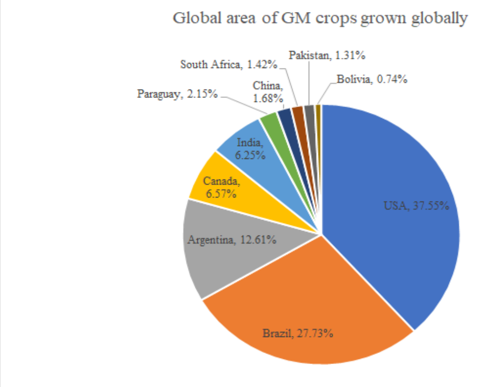

Fig. 2. Percentage of total area GM crops are grown in some of the countries

7. Conclusion and Future Scope

Bt genes can create potential problems to the environment, humans and the agricultural land. From the past research, it becomes evident that the Bt gene contamination which has widely led to insect-resistant weed is troublesome. It can restrict the feeding ground of the target in organisms as seen in cases of: corn rootworm, corn earworm, and european corn borer which can have undesired environmental effects. This is caused due to the food chain of the target pest being disturbed and an increase of resistant pests which creates agricultural problems. The farmers had to face a significant yield reduction from the damage caused by Cry protein resistant worms and even though there are many strategies to reduce this resistance, not many are effective against all Bt genes. For example, the seed blend refuge works only upto a certain time before becoming ineffective against Bt resistant worms. Even though the introduction of the Bt gene affects the targeted organism directly, causing these environmental and agricultural issues, the information on its effect on non-target organisms is presently very minimal and can be regarded as negligible. The Cry protein being consumed by different animals in the food chain can be digested and excreted out, the protein does can be diluted in the food chain, hence it holds no adverse effect. Nevertheless, the investigation of the non-target organism still needs to be held further as the accumulation of Cry protein in some insects is being reported but there is not enough evidence to prove harmful. The ambiguity of the danger of Cry protein also persists in whether it is dangerous for human consumption. With current evidence it can be considered safe, since there is no proof for potential allergens from Bt corn. These concerns regarding the safety of the technology on the environment and humans, along with the ethical concern need to be noted with care and alacrity. The decision-making bodies and plant developers, therefore, still have to improve on accessing the GMOs by being attentive towards various aspects with consistency and transparency.

References

[1] Akin, H., Yeo, S. K., Wirz, C. D., Scheufele, D. A., Brossard, D., Xenos, M. A., & Corley, E. A. (2019). Are attitudes toward labeling nano products linked to attitudes toward GMO? Exploring a potential 'spillover' effect for attitudes toward controversial technologies. Journal of Responsible Innovation, 6(1).

[2] Bailleul, D., Ollier, S., &Lecomte, J. (2016, June). Genetic Diversity of Oilseed Rape Fields and Feral Populations in the Context of Coexistence with GM Crops.

[3] Carzoli, A. K., Aboobucker, S. I., Sandall, L. L., Lübberstedt, T. T., &Suza, W. P. (2018). Risks and opportunities of GM crops: Bt maize example. Global Food Security, 19, 84-91.

[4] Casacuberta, J. M., Devos, Y., Jardin, P. d., Ramon, M., Vaucheret, H., &Nogué, F. (2015, March). Biotechnological uses of RNAi in plants: risk assessment considerations. Trends in Biotechnology, 33(3), 145-147.

[5] Clair, C. R. S., Head, G. P., &Gassmanm, A. J. (2020). Western corn rootworm abundance, injury to corn, and resistance to Cry3Bb1 in the local landscape of previous problem fields. Western corn rootworm abundance, injury to corn, and resistance to Cry3Bb1 in the local landscape of previous problem fields.

[6] Clair, C. R. S., Head, G. P., & Gassmann, A. J. (2020, August 4). Comparing Populations of Western Corn Rootworm (Coleoptera: Chrysomelidae) in Regions with and Without a History of Injury to Cry3 Corn. Journal of Economic Entomology, 113(4), 1839–1849.

[7] Dively, G. P., Huang, F., Oyediran, I., Burd, T., &Morsello, S. (2019, June 3). Evaluation of gene flow in structured and seed blend refuge systems of non-Bt and Bt corn. Journal of Pest Science, 439–447.

[8] Do, D. (2012). Rethinking risks: should socioeconomic and ethical considerations be incorporated into the regulation of genetically modified crops? Havard Journal of Law and Technology, 26(26).

[9] Dunn, S. E., Vicini, J. L., Glenn, K. C., Fleischer, D. M., & Greenhawts, M. J. (2017, September 1). The allergenicity of genetically modified foods from genetically engineered crops A narrative and systematic review. 119(3), 214-222.

[10] The Economic TImes. (2021, August). Only Bt. Cotton is approved GM Crop: Narendra Singh Tomar https://economictimes.indiatimes.com//news/economy/agriculture/only-bt-cotton-is approved-gm-crop-narendra-singh tomar/articleshow/70044566.cms?utm_source=contentofinterest&utm_ medium. https://economictimes.indiatimes.com/wealth/plan/learn-wealth-management-from-theexperts-at-the-6th-india-wealth-management-conference/articleshow/85270698.cms

[11] Fausther-Bovendo, H., &Kobinger, G. (2021). Plant-made vaccines and therapeutics. 373(6556), 740-741.

[12] Gassmann, A. J. (2021). Resistance to Bt Maize by Western Corn Rootworm: Effects of Pest Biology, the Pest–Crop Interaction and the Agricultural Landscape on Resistance (K. M. Mikac, Ed.). Insects, 12(2).

[13] Gassmann, A. J., Petzold-Maxwell, J. L., Clifton, E. H., Dunbar, M. W., Hoffmann, A. M., Ingber, D. A., &Keweshan, R. S. (2014). Field-evolved resistance by western corn rootworm to multiple Bacillus thuringiensis toxins in transgenic maize. PNAS.

[14] Gassmann, A. J., Petzold-Maxwell, J. L., Keweshan, R. S., & Dunbar, M. W. (2011, July 29). Field-Evolved Resistance to Bt Maize by Western Corn Rootworm.

[15] Gassmann, A. J., Shrestha, R. B., Kropf, A. L., Clair, C. R. S., &Brenizer, B. D. (2019). Field-evolved resistance by western corn rootworm to Cry34/35Ab1 and other Bacillus thuringiensis traits in transgenic maize. Pest Management Science, Volume 76(1), 268- 276.

[16] Genetically modified organisms: the case for new regulations. (2013, August 27). GOV.UK. https://www.gov.uk/government/publications/genetically-modified-organisms-the-case for-new-regulations

[17] Goodman, R. E., Ebisawa, M., Ferreira, F., Sampson, H. A., Ree, R. v., Vieths, S., Baumert, J. L., Bohle, B., Lalithambika, S., Wise, J., & Taylor, S. L. (2016, Febuary 17). AllergenOnline: A peer-reviewed, curated allergen database to assess novel food proteins for potential cross-reactivity. Molecular Nutrition Food Research, 60(5), 1183-1198.

[18] Gudeta, T. B. (2019). Risk Assessment and Gene Flow: Implication for Genetic Resource Conservation (A Review Article), 73. 10.7176/ALST

[19] The Hindu Business Line. (2021, Augusr India to maintain top rank in world cotton production till 2030). Comodities. https://www.thehindubusinessline.com/markets/commodities/india-to-maintain-top-rankin-world-cotton-production-till-2030/article35699684.ece

[20] Huang, F. (2020, December 31). Dominance and fitness costs of insect resistance to genetically modified Bacillus thuringiensis crops. GM Crops & Food, 192-211. Taylor and Francis Online.

[21] Hughson, S. A., & Spencer, J. L. (2015, January 21). Emergence and Abundance of Western Corn Rootworm (Coleoptera: Chrysomelidae) in Bt Cornfields with Structured and Seed Blend Refuges. Journal of Economic Entomology, 108(1), 114-125.

[22] Jakka, S. R.K., Shrestha, R. B., & Gassmann, A. J. (2016). Broad-spectrum resistance to Bacillus thuringiensis toxins by western corn rootworm (Diabroticavirgiferavirgifera). scientific reports.

[23] Jurat-Fuentes, J. L., Heckel, D. G., &Ferré, J. (2021). Mechanisms of Resistance to Insecticidal Proteins from Bacillus thuringiensis. Annual Review of Entomology, 66, 121- 140.

[24] Khajuria, C., Ivashuta, S., Wiggins, E., Flagel, L., Moar, W., Pleau, M., Miller, K., Zhang, Y., Ramaseshadri, P., Jiang, C., Hodge, T., Jensen, P., Chen, M., Gowda, A., McNulty, B., Vazquez, C., Bolognesi, R., Haas, J., Head, G., & Clark, T. (2018, May 14). Development and characterization of the first dsRNA-resistant insect population from western corn rootworm, DiabroticavirgiferavirgiferaLeConte.

[25] Kuzama, J. (2018). Regulating Gene-Edited Crops. Issues in Science and Technology, 35.

[26] Landry, H. (2015, August 10). Challenging Evolution: How GMOs Can Influence Genetic Diversity. https://sitn.hms.harvard.edu/flash/2015/challenging-evolution-how-gmos-can-influence genetic-diversity/

[27] Li, Y., Zhang, Q., Liu, Q., Meissle, M., Yang, Y., Wang, Y., Hua, H., Chen, X., Peng, Y., &Romeis, J. (2017, October). Bt rice in China — focusing the nontarget risk assessment. Plant Biotechnology Journal, 15(10), 1340-1345.

[28] Meissle, M., &Romeis, J. (2017, April 4). Transfer of Cry1Ac and Cry2Ab proteins from genetically engineered Bt cotton to herbivores and predators. Insect Science, 25(5), 823- 832.

[29] Meissle, M., Zünd, J., Waldburger, M., & Romeis, J. (2014, July 31). Development of Chrysoperlacarnea (Stephens) (Neuroptera: Chrysopidae) on pollen from Bt-transgenic and conventional maize. Science Reports.

[30] National Academies of Sciences, Engineering, and Medicine,Division on Earth and Life Studies, Board on Agriculture and Natural Resources, Committee on Genetically Engineered Crops: Past Experience and Future Prospects. (2016). Genetically Engineered Crops: Experiences and Prospects. National Academic Press Washington, DC. https://books.google.co.in/books?hl=en&lr=&id=S6A4DwAAQBAJ&oi=fnd&pg=PR1& ots=7rRD5Ne9oX&sig=7Hr q9YuiMI14iW9mAcXeAwNoh4&redir_esc=y#v=onepage&q&f=false

[31] Ober, H. (2021, July). Modified yeast inhibits fungal growth in plants External application could reduce agricultural reliance on fungicides. https://news.ucr.edu/articles/2021/07/14/modified-yeast-inhibits-fungal-growthplants#:~:text=About%2070%2D80%25%20of%20crop,ways%20to%20combat%20fung al%20diseases

[32] Papadopoulou, N., Devos, Y., Lanzoni, A., Waigmann, E., & Álvarez-Alfageme, F. (2020, April 21). Risk Assessment Considerations for Genetically Modified RNAi Plants: EFSA’s Activities and Perspective.

[33] Paula, D. P., &Andow, D. A. (2019, Febuary). Uptake and bioaccumulation of Cry toxins by an aphidophagous predator. Environmental Pollution, 209, 164-168.

[34] Peterson, J. A., Obrycki, J. J., & Harwood, J. D. (2016, May 20). Spiders from multiple functional guilds are exposed to Bt-endotoxins in transgenic corn fields via prey and pollen consumption. Biocontrol Science and Technology, 26(9), 1230-1248.

[35] Rizwan M., Hussain, Shimelis, Hameed, Atif, Azhar, Qamar, & Asif. (2019, June 19). Gene flow from major genetically modified crops and strategies for containment and mitigation of transgene escape: a review. Applied ecology and environmental research, 11191-11208.

[36] Romeis, J., Naranjo, S. E., Meissle, M., & Shelton, A. M. (2019, March). Genetically engineered crops help support conservation biological control. Biological Control, 130, 136-154. Elsevier.

[37] Ruiz, M. T. G., Knapp, A. N., & Garcia-Ruiz, H. (2018, November 2). Profile of genetically modified plants authorized in Mexico. GM Crops & Food, 9(3).

[38] Samineni, S., Sen, M., Sajja, S. B., & Gaur, P. M. (2020, February). Rapid generation advance (RGA) in chickpea to produce up to seven generations per year and enable speed breeding. The Crop Journal, 8(1), 164-169.

[39] Santis, B. D., Stockhofe, N., Wal, J.-M., Weesendorp, E., ean-Paul, e.-P., Dijk, J. v., Kok, E., Giacomo, M. D., Einspanier, R., Onori, R., Brera, C., Bikker, P., Meulen, J. v. d., & Gijs, K. (2018). Case studies on genetically modified organisms (GMOs): Potential risk scenarios and associated health indicators. Food and Chemical Toxicology, 117, 36-65.

[40] Shanahan, J., Scheufele, D., & Lee, E. (2001). Article Navigation Trends: Attitudes about Agricultural Biotechnology and Genetically Modified Organisms. Oxford Academic, 65(2).

[41] Shukla, M., Al-Busaidi, K. T., Trivedi, M., & Tiwari, R. K. (2018, October 22). Status of research, regulations and challenges for genetically modified crops in India. GM Crop & Food, 9(4).

[42] Signorini, A. M., Abratti, G., Grimi, D., Machado, M., Bunge, F. F., Parody, B., Ramos, L., Cortese, P., Vesprini, F., Whelan, A., Araujo, M. P., Podworny, M., Cadile, A., & Malacarne, M. F. (2018, May 25). Management of Field-Evolved Resistance to Bt Maize in Argentina: A Multi-Institutional Approach. Biosafety of Genetically Modified Organisms, 2.

[43] Singh, R., & Singh, G. S. (2017, September). Traditional agriculture: a climate-smart approach for sustainable food production. Energy, Ecology and Environment, 2, 296-316.

[44] Smyth, S. J. (2020, April). The human health benefits from GM crops. Plant Biotechnology, 18(4), 887–888.

[45] Sutton, T. A., Assa'ad, A. H., Steinmetz, C., & Rothenberg, M. E. (2003, November 1). A negative, double-blind, placebo-controlled challenge to genetically modified corn. Journal of Allergy and Clinical immunology, 112(5), 1011-1012.

[46] Svobodová, Z., Shu, Y., Habuštová, O. S., Romeis, J., &Meissle, M. (2017, July 19). Stacked Bt maize and arthropod predators: exposure to insecticidal Cry proteins and potential hazards. Biological Sciences.

[47] Tian, J.-C., Long, L.-P., Yao, J., Romesis, J., & Shelton, A. M. (2015, November 12). Bt crops benefit natural enemies to control non-target pests. Scientific Reports, 5.

[48] Tian, J.-C., Romeis, J., Liu, K., Zhang, F., Zheng, X.-S., Xu, H.-X., Chen, G.-H., He, X.- C., & Lu, Z.-X. (2017, August 10). Assessing the effects of Cry1C rice and Cry2A rice to Pseudogonatopusflavifemur, a parasitoid of rice planthoppers. Scientific Reports.

[49] Tinsley, N. A., Estes, R. E., & Grey, M. E. (2012). Validation of a nested error component model to estimate damage caused by corn rootworm larvae. Journal of Applied Entomology, 137(3), 161-169.

[50] United States Environmental Protection Agency. (2015, 3 08). Current & Previously Registered Section 3 Plant-Incorporated Protectant (PIP) Registrations Resources Plant Incorporated Protectants (PIPs) PIP labels and regulatory approvals via PPLS by entering the EPA registration number 2010 Bt Corn Reregistration Ter. EPA United States Environmental Protection Agency. Retrieved 8 7, 2021, from https://archive.epa.gov/pesticides/reregistration/web/html/current-previously-registered section-3-plant-incorporated.html

[51] U.S. Environmental Protection Agency. (2010). Biopesticides registration action document. https://www3.epa.gov/pesticides/chem_search/reg_actions/pip/cry3bb1-brad.pdf

[52] Vyavhare, S. S., Porter, P., & Glass, S. (2021, January 11). Emergence of Corn Earworm, Helicoverpazea, from Vip3a Seed Blend Versus Structured Refuge Ears of Maize. Southwestern Entomologist, 45(4).

[53] Wang, T., Zhang, H., & Zhu, H. (2019, June 15). CRISPR technology is revolutionizing the improvement of tomato and other fruit crops. Suburban Health Inequality. CRISPR technology is revolutionizing the improvement of tomato and other fruit crops.

[54] Ward, B. J., Marakov, A., Séguin, A., Pillet, S., Trépanier, S., Dhaliwall, J., Libram, M. D., Vesikari, T., & Landry, N. (2020, November). Efficacy, immunogenicity, and safety of a plant derived, quadrivalent, virus-like particle influenza vaccine in adults (18–64 years) and older adults (≥65 years): two multicentre, randomised phase 3 trials. 396(10261), 1491-1503.

[55] Vyavhare, S. S., Porter, P., & Glass, S. (2021, January 11). Emergence of Corn Earworm, Helicoverpa zea, from Vip3a Seed Blend Versus Structured Refuge Ears of Maize. Southwestern Entomologist, 45(4).

[56] Wang, T., Zhang, H., & Zhu, H. (2019, June 15). CRISPR technology is revolutionizing the improvement of tomato and other fruit crops. Suburban Health Inequality. CRISPR technology is revolutionizing the improvement of tomato and other fruit crops

[57] Ward, B. J., Marakov, A., Séguin, A., Pillet, S., Trépanier, S., Dhaliwall, J., Libram, M. D., Vesikari, T., & Landry, N. (2020, November). Efficacy, immunogenicity, and safety of a plant-derived, quadrivalent, virus-like particle influenza vaccine in adults (18–64 years) and older adults (≥65 years): two multicentre, randomised phase 3 trials. 396(10261), 1491-1503.

[58] Wechsler, S., & Smith, D. (2018, April 16). Has Resistance Taken Root in U.S. Corn Fields? Demand for Insect Control. American Journal of Agricultural Economics, 100(4), 1136-1150.

[59] Yang, F., Kerns, D. L., Brown, S., Head, G. P., & Huang, F. (2017, June 5). Pollen contamination in seed mixture increases the dominance of resistance to Bt maize in Spodoptera frugiperda (Lepidoptera: Noctuidae). Pollen contamination in seed mixture increases the dominance of resistance to Bt maize in Spodoptera frugiperda (Lepidoptera: Noctuidae), 73(11), 2379-2385.

[60] Zetterberg, C., &Björnberg, K. E. (2017). Time for a New EU Regulatory Framework for GM Crops? Journal of Agricultural and Environmental Ethics.

[61] Zhao, Z., Li, Y., Xiao, Y., Ali, A., Dhiloo, K. H., Chen, W., & Wu, K. (2016, June 6). Distribution and Metabolism of Bt-Cry1Ac Toxin in Tissues and Organs of the Cotton Bollworm, Helicoverpaarmigera. 212.