Novelty Journals

ISSN 2394-966X

International Journal of Novel Research in Life Sciences
Vol. 8, Issue 5, pp: (41-48), Month: September - October 2021, Available at: www.noveltyjournals.com

Resistance Evolution in GMOs and its Management Strategies: The Case of Transgenic Plants

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Abstract: **Genetic engineering and transformation have played a key role in crop improvement by introducing beneficial foreign genes or silencing the expression of endogenous genes in crop plants. Genetically modified pest resistance crops have been attracting attention recently as an alternative to chemical pesticides. However, the most important adverse characteristic of genetically modified crops is the capacity of insects and weeds to develop resistance to insecticide and herbicide. The transfer of genes from herbicide-resistant crops to wild relatives leads to creation of superweed; vector mediated horizontal gene transfer and recombination creates new pathogenic bacteria and virulent strains of the virus. Resistance management strategy is a mechanism used to delay or prevent the occurrence of resistance evolution by avoiding resistance, delaying resistance and making resistant populations revert to susceptibility. In this review, we present a detail update on the current status of the reasons for resistance evolution in transgenic crops. We also discuss issues on the risks associated with the widespread adoption of transgenic crops and strategies to manage resistance evolution.**

Keywords: **phrases: genetic engineering, herbicide, insecticide, resistance management, superweed.**

1. INTRODUCTION

Genetically modified (GM) crops are a crop whose genome have been altered using genetic engineering techniques to improve the existing traits or for introduction of a new trait that is not found naturally in the given crop species. The capacity to introduce a gene of interest from a distant species into plants has given breeders access to a whole new gene resource pool in their quest to improve crop survival, productivity, and products (Kumar *et al*., 2020). The plants produced by the insertion of specific regions of foreign nucleic acid/gene sequence into its genome using transformation methods such as Agrobacterium-mediated transformation or direct gene transfer are known as transgenic plants (Griffiths *et al*. 2005). The inserted gene, also known as transgene, may come from an unrelated plant, bacteria, virus, fungus, or an animal species. Thus, the advent of genetic transformation overcomes the major limitation of conventional plant breeding in which sexual compatibility between species is prerequisite to cross them. (Singh *et al*., 2014).

Genetically modified crops are designed to acquire many useful quality attributes such as pest resistance, herbicide resistance, disease resistance, high nutritional quality and yield potential. Another importance of genetic modification is their application as bioreactors for the production of nutraceuticals, therapeutic agents, antigens; monoclonal antibody fragments, biopolymers (Smita, 2013; Kumar *et al*., 2020). For example, insect pest resistance has mostly been obtained by using genes obtained from soil bacterium *Bacillus thuringiensis* (Bt). *B. thuringiensis* encodes for the Bt toxin which is active against Lepidopteran insects and has been introduced mostly in cotton and maize against many insects (Tabashnik and Carrière, 2017; Carrière and Tabashnik *et al*., 2015). This bacterium produces gram-endotoxin protein which is toxic for certain insects.

The transgenic plant that contains Bt toxin withstands the repeated heavy infestation of target insect pests that totally devastate non transgenic plants (Salehi *et al*., 2008). Virus resistance is mostly achieved by introducing gene sequences derived from pathogenic viruses into the crop genome using gene silencing, antisense RNA and RNAi techniques (Zhao *et al*., 2020). Herbicide resistance genes for a wide range of herbicides have been recognized, characterized and transferred into a wide range of plants leading to a rapid progress in the development of herbicide resistance transgenic plants (Gaines *et al*., 2020). For example, acetohydroxy acid synthase has been introduced into a variety of plants, which make plants that express this enzyme resistant to multiple types of herbicides (Ordonio *et al*., 2016).

Furthermore, genetic modification has made it possible to engineer each crop with new insecticidal genes and this is promising the world safer pesticides, reduction in chemically intensive farming and a more sustainable agriculture. However, there are concerns that many scientists have expressed regarding the possible environmental risks of genetically engineered organisms (Lovei and Bøhn 2010; Kumar *et al*., 2020). The most serious ecological risks posed by the commercial-scale use of transgenic crops are the spread of transgenic crops threatening crop genetic diversity by simplifying cropping systems and promoting genetic erosion, potential transfer of genes from herbicide resistant crops to wild or semi-domesticated relatives thus creating superweeds, emergence of volunteers weeds, vector-mediated horizontal gene transfer and recombination to create new pathogenic bacteria, vector recombination to generate new virulent strains of virus, and insect pests will quickly develop resistance to crops with Bacillus thuringiensis (Bt) toxin (Kumar *et al*., 2020). This review presents a brief overview on the risks associated with the evolution of resistance in genetically modified plants and strategies to manage the problem.

2. RESISTANCE EVOLUTION

2.1. Antibiotics resistance

In the processes of genetic engineering, antibiotics are used as selection markers, to distinguish successfully transformed bacteria from non-transformed one (*Zhang et al., 2016)*. The large presence of antibiotic resistance genes in the environment and soil, as well in the food eaten by animals and humans, could transfer the antibiotic resistance marker genes to bacteria in the guts of animals or humans, or to bacteria in the environment (Tabashnik ,1994; Ricroch *et al*., 2011; Gilbert, 2013).

The research findings indicated that soil samples collected from GM plant cultivation field contains the transgene DNA (Lerat *et al*., 2005; Lerat *et al*., 2007). Some plant residues (roots, stems, leaves, and pollen grains) remain in the field after harvest and decayed by mechanical and chemical degradation or by microbial action. As a result, the plant cellular contents, including DNA released in to the environment (Gay and Gillespie, 2005). The fragments of DNA that are large enough to include an open reading frame of the size of an antibiotic-resistance marker gene can survive in the field or in the animal gut and can be incorporated in to bacteria (Gay and Gillespie, 2005).

Some soil bacteria are able to develop natural competence to acquire novel genetic elements through natural transformation. (Lorenz and Wackernagel,1994; Dubnau, 1999). Studies have demonstrated that naturally competent bacterial cells can take up extracellular DNA containing antibiotic resistance genes carried by GM plants and incorporate the DNA into their genomes via homologous recombination (Nielsen *et al*., 1997; Gebhard *et al*., 1999; de Vries *et al*., 2001).

It is speculated that the consumption of GM foods containing antibiotic resistance marker gene by humans and animals may lead to transfer of these genes from GM food to microflora in the gut of humans and animals or to the pathogens in the environment transforming them into strains that are resistant to antibiotic therapy (Kaeppler, 2000; Verma, 2013). Even though there are possibilities for horizontal gene transfer from GMOs to other organisms, naturally horizontal gene transfer occurs at a very low rate (Ma *et al*., 2003). Therefore, it is very important to conduct risk analysis, prediction, prevention and monitoring of the negative impact of GM products.

2.2 Herbicide Resistance

Transgenic breeding through genetic engineering for herbicide tolerance, insect resistance, abiotic stress tolerance, disease resistance and nutritional involves introduction of beneficial foreign gene(s) or turning off the expression of endogenous gene(s) in crops (Rani and Usha, 2013; Kumar et al., 2020). Barragán-Ocaa *et al*., (2019) report that around 32 crops have been approved for production in various parts of the world. However, widespread adoption of transgenic crops containing foreign genes has been hampered by concerns about human toxicity and allergenicity, as well as potential environmental risks such as gene flow, adverse effects on non-target organisms, and weed and insect resistance evolution (Owen and Zelaya, 2005; Barragán-Ocaa *et al*., 2019; Kumar *et al*., 2020). One of the most common features added into genetically modified organisms, or GMOs, is herbicide resistance. This was done in order to provide new methods for managing and controlling weeds in crop fields, which may result in weed population shifts, the evolution of herbicide-resistant weed populations, and the turning of weeds into uncontrollable superweeds and volunteer weeds. (Guanting and Yingwu, 2001; Owen and Zelaya, 2005).

Examples of herbicide-resistant weeds include populations of horseweed (Conyza canadensis (L) Cronq) resistant to N-(phosphonomethyl) glycine (glyphosate) (Owen and Zelaya, 2005; Green, 2014).

Glyphosate is the most commonly used and effective herbicide that has ever been discovered (Heap and Duke, 2018). However, the widespread use of glyphosate-resistant crops and reliance on glyphosate for weed control created unprecedented conditions for herbicide-resistant weeds to evolve (Baek *et al*., 2021). As a result of resistance development, certain herbicide resistant crops that were initially very effective at controlling weeds in combination with glyphosate herbicide are no longer as useful as they once were (Heap and Duke, 2018). glyphosate resistance has developed by a variety of methods, including single, double, and triple amino acid changes in the target-site gene, duplication of the gene encoding the target site, and others that are uncommon or nonexistent for evolved resistance to other herbicides (Heap and Duke, 2018; Baek *et al*., 2021).

2.3. Insecticide resistance

For nearly a century, insecticidal proteins from the bacterium *B. thuringiensis* have been used to control several agriculturally significant insect pests through sprays, and genetically altered crops since 1996. (Sanahuja *et al*., 2011). Globally planted Transgenic crops that produce insecticidal proteins from *B. thuringiensis* for insect control have increased in number from 1 million in 1996 to more than 108 million in 2019 (ISAAA, 2021).

Bt crops can suppress insects and reduce the need for insecticide sprays, thereby providing economic and environmental benefits but these benefits can be reduced or even eliminated by the evolution of resistance in insect's pests (Dively *et a*l., 2018, Qi *et al*., 2021, Tabashnik *et al*., 2021). The extensive and repetitive use of *B. thuringiensis* crops on a large-scale places' enormous selection pressure on insect pests, raising the risk of insect pests developing resistance to Bt crops' insecticidal effect. (Tabashnik and Carrière, 2019, Wei *et al*., 2019). The effectiveness of nine Cry proteins has been reduced in 21 cases of field-evolved resistance (Calles-Torrez *et al*., 2019, Tabashnik and Carrière, 2020). Practical resistance to Bt crops has evolved in about nine important pests, which is defined as field-evolved resistance with practical consequence for insect pest control (Calles-Torrez *et al*., 2019; Smith *et al*., 2019; Tabashnik and Carrière, 2019). Organic farmers are concerned because they utilize Bt as a natural insecticide. Due to field-evolved resistance, the effectiveness of Bt crops has deteriorated, and management techniques should be developed to control or minimize its occurrence.

3. RESISTANCE MANAGEMENT

A practicable resistance management strategy should either delay or prevent the occurrence of resistance evolution. This means that the resistance management strategy should not place undue burdens on farmers and other parties who will implement the strategy or such a burden should be at least partially offset by implementation incentives. In other words, the costs associated with implementing resistance management must be considered in setting the resistance strategy. Although preventing resistance and control failures would seem the more sustainable goal, prevention requires active management or evolutionary selection pressures against resistance allele in a population (Gould and Tabashnik, 1998). Generally, there are three goals of resistance management: avoiding resistance where and if possible, delaying resistance as long as possible, and making resistant populations revert to susceptibility (Croft, 1990).

The release of transgenic crops may give rise to potential ecological risks, one of which is that some transgenes (mainly herbicide-resistant genes) would spread into related weeds or wild species by pollination, turning them into uncontrollable superweeds (Gressel, 1999; Guanting and Yingwu, 2001)

3.1.1 Transgenic mitigation

Gene flow occurs most often between closely related species where there are few genetic barriers. There are some transgenic crops where crop–crop and crop–weed gene flow cases have been reported (Gressel, 2012; Zhang *et al*., 2020). The goal of transgenic mitigation, keeping the transgene of choice within the crop and thus preventing it from introgressing into related weeds, crop varieties, and wild species is based on three principles. (i) Tandem constructs of genes act genetically as tightly linked genes, and their segregation from each other is exceedingly rare. The desired transgenic can be paired with genes that make hybrid offspring or volunteer weeds less competitive against crops, weeds, and wild species. (ii) There are features that are either neutral or favorable for crops but are harmful to weeds, such as volunteer weeds and wild species. (iii) If a crop's desired gene is flanked by transgenic mitigation genes in a tandem construct, the overall effect would be negative if it spread to weeds (Gressel, 1999). Transgenic mitigation could benefit from genes that prevent seed shattering or secondary dormancy, as well as genes that shrink the recipient (Gressel, 1999; Daniell, 2002).

3.1.2 Gene pyramiding and silencing

Cry and vegetative insecticidal protein (Vip) insecticidal proteins are designed to reduce resistance evolution in pyramided crops that produce two or more different Bt toxins. Pyramided plants have long been utilized to manage insects and are critical in delaying insect resistance (Carrière *et al*., 2015). To manage resistance evolution in Bt crops, researchers designed a transgenic cotton (Bollgard III (BGIII)) that expresses three Bt toxin genes (Cry1Ac, Cry2Ab, and Vip3A).

Resistance to insect pests is being improved through plant-mediated RNAi of critical pest genes involved in defense, detoxification, digesting, and development. currently RNAi technology or RNAi pyramided with Bt genes has been used to generate insect-resistant transgenic crops (Wu et al. 2016; Ni *et al*. 2017).

Ni *et al* (2017) developed a pyramid of cotton containing Bt and RNAi, and found excellent results against cotton bollworm, and also substantially delayed resistance as compared with using Bt alone. Pyramiding of multiple RNAi expression cassettes against various essential genes involved in the defense, detoxification, digestion, and development of cotton pests will successfully obtain favorable agronomic characters for crop protection and production. The development of transformable synthetic chromosomes with many unique Bt toxins and RNAi to knock down various key target genes of pests is known as multiple gene pyramiding and silencing (MGPS) (Ren *et al*. 2019). Due to the synergistic impact of high concentrations of Bt toxins and RNAi(s), as well as compliance with appropriate refuge, the evolution of insect resistance in transgenic crops will be delayed or suppressed.

3.1.3 High –dose /Refuge Strategy

This technique requires Bt plants to produce enough Bt protein to kill 99 % of susceptible individuals and 95% of the heterozygous individual for resistance alleles (Tabashnik and Carrière, 2017, Reisig and Kurtz, 2018), rendering resistance functionally recessive.

Refuge involves the planting of non-Bt host plants that are planted close or adjacent to, or within, the Bt crop fields (Tabashnik and Carrière, 2017). Refuge areas provide a reservoir of susceptible target insects that will mate with those rare resistant individuals emerging from Bt crop fields. If resistance is a recessive trait, heterozygous offspring produced by such crosses will not be able to survive on Bt plants. The ideal size and structure of refuge zones, as well as their distance from Bt crop fields, is determined by the target insect pest's population size, feeding patterns, and dispersal ability (Hutchison *et al*., 2010, Tabashnik and Carrière, 2017, Carrière *et al*., 2020).

In general, it is recommended that a 20-50% refuge area be planted with non-GMO varieties. To avoid the development of insect pest populations resistant to Bt corn, it is recommended that a minimum of 20% of the area be planted with conventional varieties; and in fields where Bt corn is planted where cotton has previously been cultivated, at least 50% of the area must be planted with conventional varieties of corn (Cannon 2000). This is an effective way that can lead to the delay in the development of resistance in pests against Bt-crops that can assist in using the same genes for a longer period (Carrière *et al*. 2016).

3.1.4 Release of Sterile Insects

The release of sterile insect methods is well-known for decades, which has been successfully used in different insect pests (Krafsur, 1998 and Tabashnik *et al*., 2010). The use of this strategy to reduce pests resistant to transgenic crops was initially described in a computer simulation research, which demonstrated that releasing sterile moths suppressed the resistance of the pink bollworm in Bt cotton (Tabashnik *et al*., 2010).

Introductions of MS-engineered P. xylostella males into wild-type populations led to rapid pest population decline, and then elimination. In a separate experiment on broccoli plants, the release of relatively low level of MS males in combination with broccoli expressing Cry1Ac (Bt broccoli) suppressed population growth and delayed the spread of Bt resistance (Harvey-Samuelet al., 2015).

3.1.5 Seed Mixtures

Seed mixtures (also known as 'refuge-in-a-bag' or RIB) yielding a random mixture of Bt plants and non-Bt plants side-by-side at intervals fields are planted to delay pest resistance to Bt corn pyramids (Carrière *et al*. 2016). Seed mixtures solve the matter of farmers not yielding with block refuge needs. However, results from modeling and small-scale experiments indicate that if larvae move between Bt and non-Bt plants, seed mixtures could accelerate the evolution of resistance by reducing the survival of vulnerable insects and also the effective refuge size, or by increasing the survival of heterozygotes relative to vulnerable homozygotes, thereby increasing the dominance of resistance in seed mixtures relative to blocks of Bt crops (Carrière et al. 2016).

In China, millions of growers enforced a unique seed mixture strategy by planting second-generation seeds from crosses between Bt and non-Bt cotton, that yields a refuge of twenty fifth non-Bt plants randomly interspersed at intervals fields of Bt cotton (Wan *et al*., 2017). Analysis of eleven years of field watching information from six provinces implies that this approach delayed or even reversed *Pectinophora gossypiella* resistance to single-toxin Bt cotton whereas sustaining pest suppression (Wan *et al*., 2017).

3.1.6 Integrated Pest Management (IPM)

Integrated pest management is a strategy for pest control that considers the use of all economically available pest control techniques without relying on only one of these (Lamichhane *et al*., 2017). It is assumed that resistance is less likely to evolve to two control methods simultaneously than to only one method. Thus, with two more methods, resistance to the combination will be delayed more than using each individually in a temporal or spatial arrangement. Crop rotation, seed combination, a high-dose/Refuge strategy, and the use of additional management techniques can all be used as insurance if one of them fails. Transgenic crops based on multiple genes pyramiding and silencing (MGPS) coupled with refuge can be an effective and smart way to control insect pests. (Neppl, 2000; Carrière *et al*., 2019; Carrière *et al*., 2020).

4. CONCLUSION

In conclusion, genetic engineering of new traits into crops has advanced agriculture, leading to higher yields, new products and resistance to insect pests, diseases and herbicides. Although genetic transformation provides plants with vital traits mentioned, a large-scale introduction and widespread adoption of transgenic crops carrying foreign genes will result in intense selection pressures that will rapidly lead to the development of resistance evolution. The major environmental concerns associated with transgenic crops, but not limited, antibiotic, herbicide and pesticide resistance evolution. To overcome these challenges, various resistance management approaches have been practiced for years with the objective of delaying or preventing the evolution of resistance. However, integrated resistance management strategies involving two or more control methods have found to be more effective than when a single tactic is used alone.

Conflict of interest

The author declared that no conflict of interest

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