



ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(5): 331-334
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www.thepharmajournal.com

Received: 08-02-2022

Accepted: 16-04-2022

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Genetic pollution in plants: A review

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Abstract

A Genetically Modified organism is an animal, plant or microbe whose DNA has been altered using genetic engineering techniques. In the recent era genetically modified crops are widely used in cultivation. GMO contains genes from different sources like animals, microbes or different plant species and they are not present in recipient crop in nature before. Genetic pollution occurs due to the unintentional process of genetically modified organisms (GMOs) dispersing their genes into the natural environment by breeding with wild and conventional plants. The process of dispersing genes in nature is called gene escape because it happens unintentionally. Worldwide the evidences of transgene escape have been found in cotton, maize, soybean, oilseed rape, rice, and wheat etc. Cross-pollinated crops spread their pollens to long distances from their sources and pollinate their wild forms or wild relatives. Most cases are due to pollen-mediated gene transfer in related species of the same crop. Genetic pollution affects our natural ecosystem as well as non-GM crops and traditional cultivars. Many measures to avoid transgenic escape are available like isolation distance, GM-free zones and many biotechnological approaches. These approaches are essential to save our conventional lines, wild relatives, wild forms and germplasm.

Keywords: Genetic pollution, gene escape, gene flow, genetically modified organisms, transgene

Introduction

In 2019, the 24th year of commercialization of GM crops, 190.4 million hectares of GM crops were planted by up to 17 million farmers in 29 countries. The USA remained as the top producer of GM crops globally, which planted 71.5 million hectares in 2019. Soybean (91.9 mha), corn (60.9 mha), cotton (25.7 mha) and canola (10.1 mha) are the major GM crops grown commercially in the world. (ISAAA, 2019). India landed on the fifth spot, with 11.9 million hectares. (ISAAA, 2019).

The term genetic pollution was popularized by environmentalist *Jeremy Rifkin* in his 1998 book *The Biotech Century* (Rifkin, Jeremy). While intentional cross-breeding between two genetically distinct varieties is described as hybridization with the subsequent introgression of genes, Rifkin used genetic pollution to describe the risks that might occur due to the unintentional process of genetically modified organisms (GMOs) dispersing their genes into the natural environment by breeding with wild and conventional plants. Genetic pollution accounts to the uncontrolled spread of genetic information into the genomes of organisms in which such genes are not present in nature (Zaid, *et al.*). Genetically engineered (GE) plants contain genes which have been transferred from unrelated species. These may come from bacteria, viruses, other plants or even animals. If these 'foreign' genes are then transferred into other organisms, this causes genetic contamination or pollution of the natural gene pool (www.greenpeace.org). Unlike other forms of pollution, genetic contamination has the potential to be a problem that multiplies as plants and microorganisms grow and reproduce. Therefore, environmental damage caused by genetically modified organisms (GMOs) cannot be confined to the original habitat in which they are first introduced. (www.greenpeace.org).

Gene flow

In simple terminology, gene flow is the movement of genetic materials (genes or alleles) from one organism to another. In population genetics, gene flow (also known as gene migration) refers to the transfer of alleles or genes from one population to another. Theoretically, there are two types of gene flow: vertical gene flow and horizontal gene flow, although the latter is commonly referred to as horizontal gene transfer. Horizontal gene flow occurs only among unrelated species, such as between plants and microbes, as well as between microorganisms. The discussion of horizontal gene flow is based more on theory than practice, since it has never been shown to occur with transgenes outside an experimentally enforced setting, even though this process is significant in the evolution of organisms.

Transgene Escape

It is a process where the transgene inserted to a GM crops has been escaped to its wild Species/neighbour crops. The principle concern about the transgene flow is the loss of potentially useful crop genetic diversity in recipient population. The evidences of transgene escape have been found in cotton, maize, soybean, oilseed rape, rice, and wheat (Baltazar *et al.*, 2015; Dong *et al.*, 2016; Londo *et al.*, 2011; Mizuguti *et al.*, 2010; Ramzan *et al.*, 2014; Serrat *et al.*, 2013) [2, 4, 11, 15, 10, 22]. These findings are not limited to a certain region of the world instead the examples reveals global dimension of the problem. Hybridization of GM plants with their conventional parents and adventitious presence of seed has been observed as expected (Rizwan *et al.*, 2019) [18].

Genetic contamination may arise

- Wild, related flora growing nearby are pollinated by a GE crop.
- Non-GE or organic crops in neighbouring fields are pollinated by the GE crop.
- A semi-wild, weed or 'feral' population of GE plants develops if the GE crop survives in the agricultural or natural environment.
- Micro-organisms in the soil or the intestines of animals eating the GE crop acquire the foreign genes. (www.greenpeace.org).

Transgene escape in major crops

Cotton

The year 1996 may be considered as a turning point in the history of agricultural biotechnology in general and crop protection in particular as three insect resistant Bt-crops, developed by Monsanto Company, had received approval for commercialization in the USA after these had satisfied the regulatory requirements. Bt-cotton was one of them along with Bt-corn and Bt-potato. It took about 14 years of intensive research and millions of dollars to develop this technology and prove its safety and benefits. Bt-cotton (Bollgard®) was incorporated with the lepidopteron specific Bollgard® Bt-gene, cry 1Ac, targeted against cotton bollworms.

In india, cotton is first genetically engineered crop which was commercially introduced in 2002. It is primarily a self-pollinated crop but 5-30% outcrossing may occur due to pollinators (Poehlman, 2013). Its pollen is large and sticky which makes pollinators potentially important in cross pollination (Van Deynze *et al.*, 2005). Due to often cross pollination GM cotton is continuously contaminating its non-GM germplasm which have superior yield and fiber quality traits required for farmer and industrialist. This threatens the use of refuges and complicates the removal of transgene from the environment if unexpected problems arise. Many studies on the level of contamination in cotton are documented. In a recent study, Ramzan *et al.* (2014) [10] reported highest rate of contamination (22% from Bt samples and 20% from non-Bt) from Faisalabad, the city of Pakistan where previously cotton was the major commercially grown crop. Heuberger *et al.* (2010) identified the potential sources of Bt contamination and demonstrated that out crossing (due to abundance of honeybees), proximity to Bt fields and human factors contribute to seed contamination in cotton. Therefore, it is necessary for cotton breeders to screen their breeding material thoroughly for the removal of genetic contamination from non-Bt.

Maize

In maize, gene flow occurs between all sexually compatible plant types, *i.e.* commercial hybrids, landraces and eventual wild relatives (Baltazar *et al.*, 2015) [2]. The cultivation of open-pollinated varieties (OPVs) along with commercial hybrids also increase rate of gene flow (Sanvido *et al.*, 2008) [20]. The synchronization of flowering in GM maize and its non-GM is important to determine the potential of pollens for gene flow by cross-hybridization (Palaudelmas *et al.*, 2008).

Soybean

To control the weeds in soyabean a foreign gene CP4 was introduced to develop herbicide resistant soybean. Due to more availability of transgenic cultivars, the contamination of conventional cultivars and unintended combination of transgenes through natural crossing is becoming a serious threat. (Rizwan *et al.*, 2019) [18]. In order to determine if there is pollen dispersal from transgenic to nontransgenic soybean plants, a field release experiment was conducted in the Cerrado region of Brazil. The greatest amount of transgenic pollen dispersion was observed in the first row, located at one meter from the central (transgenic) plot, with a 0.52% average frequency. The frequency of pollen dispersion decreased to 0.12% in row 2, reaching 0% when the plants were up to 10 m distance from the central plot (Abud *et al.*, 2007) [1].

Oilseed rape

Oilseed rape (*Brassica napus* L.) has been genetically modified to tolerate broad spectrum herbicides. Due to its ability of producing large amount of pollen, it is an ideal crop to understand the implications of transgene flow. Oilseed rape is known to exhibit different levels of outcrossing (Rizwan *et al.*, 2019) [18].

Impacts of genetic pollution

- Direct effects on non target organisms.
- Genetically modified organisms might lead the non-GM organisms to extinction.
- Impacts of transgenic crops on parasitoids.
- Unknown health consequences are a common objection GMO organisms.
- Transgene escape from these crops may lead to the development of super weeds.
- Cross pollination with the cultivated and wild type with GM species may lead to genetic contamination of the cultivated wild type which could alter local ecosystem.

Strategies to reduce genetic pollution

Physical Containment

In many cases, it is possible to significantly reduce the frequency of pollen mediated gene flow by deploying an effective strategy of physical isolation between GM pollen donors and recipients. The extent of pollen-mediated gene flow is affected by pollen flow that has a leptokurtic distribution, with most pollen grains spreading close to the pollen donors, and only a small amount moving over longer distances. For example, most maize pollen falls within about 30 m, and most rice pollen falls within a few metres, from the pollen donor. Again, the longevity of 111 pollen viability for many crop species is only a matter of minutes. For example, the imperial expectation of rice pollen longevity is less than 10 minutes, although the pollen viability of wild rice and its hybrids can be somewhat longer. Temporal (flowering time)

isolation between pollen donors and recipients can sometimes also serve as an effective strategy for physical confinement. Frequencies of pollen-mediated gene flow can vary significantly among plant species, even among different varieties of the same crop species.

Spatial isolation

Spatial isolation involves a separation zone that could be open land or fields with other plant species that serve as a pollen barrier between the GM pollen donors and recipients. Gene flow mediated by pollination should be within the range of pollen flow of a particular species, and the frequency of pollen-mediated gene flow is determined by the pollen density around the pollen recipient at particular spatial distances.

Temporal isolation

The idea of temporal isolation is to separate the flowering time of GM from non-GM crops or totally remove pollen from the GM crops. Temporal strategies involve the use of delayed plantings and crop rotation to avoid contact between GM and non-GM crops.

GM crop free zones

Areas where more genetically diversity banned for GMO. The proper deployment of GM and non-GM crops in a region or in a country can be an effective strategy to totally avoid pollen-mediated transgene flow and GM contamination/adventitious mixing

Careful transportation of seeds from GM plants

Volunteer plants

Monitor field after taking successful GM crop to avoid volunteer plant

Growing trap crops

Crop which contain transgene cover by trap crop which not contain transgene

Using pollen barriers

Planting more heightened crops around or between GM crops

Biological and molecular Containment

Sterility

Most of the times transgene flow occurs through pollens so implying the trait of male sterility can cordially reduce the problem (Daniell, 2002)^[3].

Maternal effects

One of the few techniques for containing unwanted gene flow can be transformation of genes controlling economically important gene in plastids and mitochondria (Maliga, 2004).

Incompatible genome

By targeting the genome which is less compatible to wild and weedy relatives to incorporate the transgene will make fairly less chance for gene to escape (Lu, 2003)^[13].

Seed Lethal system

Developed a repressible seed-lethal (SL) system aimed at reducing the probability of transgene introgression into a population of sexually compatible plants (Scherthner *et al.* 2003)^[21]. To evaluate the potential of this method, tobacco plants were transformed with an SL construct comprising

gene 1 and gene 2 from *Agrobacterium tumefaciens* whereby gene 1 was controlled by the seed-specific phaseolin promoter modified to contain a binding site for the Escherichia coli TET repressor (R).

The expression of this construct allows normal plant and seed development but inhibits seed germination. Plants containing the SL construct were crossed with plants containing the tet R gene to derive plant lines where the expression of the SL construct is repressed. Plant lines that contained both constructs allowed normal seed formation and germination, whereas seeds in which the SL construct was separated from the R gene through segregation did not germinate.

Cleistogamy

A modification of flower structure to promote self-pollination and is an effective means against transgene flow. It can be induced by mutation or genetic engineering.

Apomixis

The use of apomixis is most successful method to stop transgene flow and this is also modification in floral structure that can be propagated by asexual means (Gressel, 2015; Kwit *et al.*, 2011)^[5, 8]. Use of apomixis for containment of transgene has proven in GM bahia grass where transgene flow is limited to 0.2% (Sandhu *et al.*, 2010)^[19].

Genetic Use Restriction Technology (GURT)

It referred as terminator technologies that are experimental forms of genetic engineering technology that provide the means to either restrict the use of a plant variety or the expression of a trait in a plant variety by turning a genetic switch on or off. There are currently two types of GURT's under research. Genetic use restriction technologies could be used for the environmental containment of transgenic seeds (V-GURT) or transgenes (T-GURT), thus solving or marginalizing one of the greatest concerns associated with GM crops. V-GURTs may generally prevent unwanted gene flow from transgenic to non transgenic varieties (including wild relatives) because pollen carries the dominant allele of the lethal/inhibiting protein.

Zinc finger nuclease (ZFN)-mediated transgene excision from pollen

ZFN expression under the control of a pollen-specific promoter LAT52 (LAT52) creates a double strand break in the spacer region between two adjacent ZFN recognition sites (R) forming one set of ZFN sites this results in one DNA fragment containing the functional transgenes including trait and marker genes that have been excised from pollen genome and which are destroyed in the cell and the pollen genome with only one set of two adjacent ZFN recognition sites, which by itself, is non-functional.

Conclusion

- Escape of transgene from GM crop plants to non-GM and wild relatives may pose genetic pollution.
- Chances of gene escape are more when GM crops sown near by non GM crops of e species.
- Understanding of transgene escape will facilitate the sustainable and safe cultivation of GM varieties of different crops.
- Physical and biological containments are effective to control genetic pollution by mitigate gene escape.

- Therefore, to favor the GM technology, we should take into consideration the biosafety measures as well as potential techniques to contain or mitigate the transgene effect.

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