GENE FLOW FROM MAJOR GENETICALLY MODIFIED CROPS AND STRATEGIES FOR CONTAINMENT AND MITIGATION OF TRANSGENE ESCAPE: A REVIEW

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Abstract. Recent advancements in biotechnology resulted in rapid adoption of genetically modified (GM) crops in the agriculture systems. At the same time, transgene escape has also been reported and examples reveal global dimension of the problem. Pollen mediated gene flow (PMGF) is the major pathway for transgene escape. Almost all transgenes have been escaped into their Non-GM counterpart and wild relatives. Although gene flow varies between species, crops, and ecological zones/environments but intraspecific gene flow (> 10%) is not uncommon in adjacent populations. Whereas in outcrossing species, 1% gene flow at thousand meters' isolation is not unusual, and magnitude is even higher than the mutation rate. It is well documented that transgene flow is deteriorating different production systems in agriculture and famers choice to cultivate GM, conventional and organic crops. If comprehensive policy is not implemented, then in future it will be difficult to detect and remove transgenes from the environment; if unexpected problems arise.
Keywords: *biosafety, biological containment, coexistence, genetic contamination, out-crossing, pollen dispersal, transgene flow*

Introduction

Gene flow, a natural phenomenon, changes the gene frequency in population due to outcrossing of gametes, movement of individuals across countries or groups from one place to another (Goodman and Newell, 1985; Ellstrand, 2003; Cerdeira and Duke, 2006). In the current scenario of agriculture, where 189.8 million ha of GM crops were planted in 2017 with global market value of US$ 17.2 billion (ISAAA, 2017), coexistence and identity preservation among transgenic and non-transgenic cropping systems at the field level are becoming important issues (Beckie and Hall, 2008). Concerns have been identified and raised about GM plants in new environmental conditions and in response to transgene escape into wild relatives and crop to crop has

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gained much attention of plant biologists. It is not uncommon for transgenic plants to mate with their wild relatives. Spontaneous hybridization will occur among transgenic and non-transgenic plants unless the proper distances are maintained, and also the engineered plants are specifically designed to limit gene flow (Gressel, 1999; Daniell, 2002). It is not necessary that gene flow creates problem but it may enhance local genetic diversity and in part it depends on the phenotypes conferred by transgenes (Ellstrand and Hoffman, 1990; Ellstrand, 2003). Outcrossing poses negative impacts in terms of contamination in non-transgenic crops but again problem depends on new allele whether causes an increase in transgene escape or not. According to Kareiva and Marvier (2000), gene flow varies between species, within species of same crop, populations, genotypes and environments. Surprisingly, intraspecific gene flow occurs at high rate. Gene flow of more than 10% is not uncommon in adjacent populations. For outcrossing species, 1% gene flow at thousand meters isolation is not unusual and the magnitude is higher than the rate of mutation (Ellstrand, 2014). Further, adventitious mixing of GM and non-GM crops also raises the question about maintenance of different production systems in agriculture sector and famers choice to grow either GM, conventional or organic crops. Various measures have been proposed to minimize transgene escape, such as the use of refuge surrounding the GM crop in the field with barren zones, genetic methods to handicap the fitness of transgenic hybrid (Gressel, 1999; Daniell, 2002). Monitoring transgene escape has been initiated to measure the adverse impact on environment (NRC, 2002). The impact of gene flow is so high that even single gene can contaminate the population, so it is difficult to establish an effective monitoring programme (Marvier et al., 1999). This review describes the facts and thoughts behind transgene escape, sources and level of contamination in major crops, containment and mitigation strategies, their comparison, conclusion and future prospects.

Transgene escape: facts and speculations

Transgene escape is a fact and usually restricted to within species or closely related species and this is referred to as vertical gene flow. It is very rare that gene flow occurs between species, i.e. horizontal gene flow. In contrary, diagonal gene flow occurs between closely related species (Gressel, 2015). With these concerns, the genetically engineered (GE) crops have been cultivated for commercial and research purposes under some restrictions to avoid transgene escape. But after 22 years of GE crops, we failed to control gene flow in a systematic manner (Ryffel, 2014).

Convincing 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), and describes that transgene escape (*Table 1*). Transgene may not only flow in area bordering the GM field but may also happen far away. 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.

Cotton is an illustrative example, where gene flow both vertical and diagonal has been documented in several studies (*Table 1*). Transgenes in *Gossypium hirsutum* conferring insect and herbicide resistance have been escaped through pollen to conventional counterparts, *G. barbadense,* refugees and wild relatives (Heuberger et al.,

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2010). Similarly, PMGF has been reported from GE maize. Similar case is reported in Mexico, where GM corn was not allowed for commercial cultivation but transgene escape was found in landraces (Mercer and Wainright, 2008). Initially these findings were controversial but dies, good evidences for the escape of transgenes were found through comprehensive experimentations (Mercer and Wainright, 2008).

Another example is from oilseed rape, in which glyphosate resistance was found in field grown glufosinate resistant oilseed rape. The fact that in several cases stacked events that were not engineered in the laboratory identified in the field. This showed the rapidity of recombining genes between varieties of an outcrossing species (*Table 1*). In case of wheat, many studies on escape of herbicide resistance genes have been documented as a part of literature. This escape is independent off whether the resistance is created by mutagenesis or transgenic. Therefore, the risk assessment should be based on the biology of a field crop, the traits, occurrence of compatible relatives and transformation method, i.e. classical breeding or genetic engineering used for trait integration.

Table 1. *Some evidences of natural transgene escape in different crops*

From	To	Transgene escaped	Trait	Type of flow	Medium of escape	Region	Reference
Cotton *Gossypium hirsutum*	Non-*Bt.* cotton	MON-531, Cry1Ac, Cry2A	Insect resistance	Vertical	Pollen and seed	Pakistan	Ramzan et al. (2014)
	Gossypium barbadense	EPSPS	Herbicide resistance	Diagonal	Pollen	USA	Van Deynze et al. (2011)
	Refuges of non-Bt cotton	Cry1Ac	Insect resistance	Vertical	Pollen and adventitious presence of seed	Arizona USA, Tolima Colombia	Heuberger et al. (2010), Rache et al. (2013)
	Non-*Bt.* cotton	Cry1Ac and CP4 EPSPS	Insect and herbicide resistance	Vertical	Pollen	Beijing China	Yan et al. (2015)
	Wild populations	Cry1Ab/Ac, Cry2A, CP4-EPSPS and PAT/ Bar	Insect and herbicide resistance	Diagonal	Pollen	Mexico	Wegier et al. (2011)
Maize *Zea mays*	Non-GM maize	MON-810	Insect resistance	Vertical	Pollen	Slovakia, Spain	Mihalčík et al. (2012), Pla et al. (2006)
	Non-GM maize	PAT, CDC2	Herbicide tolerance	Vertical	Pollen	UK	Weekes et al. (2007)
	Non-GM maize	MON-89Ø34-3, MON-88Ø17-3, MON-ØØ6Ø3-6	Insect resistance and herbicide tolerance	Vertical	Pollen	Mexico	Baltazar et al. (2015)
	Landraces	Cry1Ab/Ac, Cry9C, CP4-EPSPS	Insect and herbicide resistance	Diagonal	Pollen	Mexico	Mercer and Wainwright (2008)
Soybean *Glycine max*	Conventional soybean	EPSPS	Herbicide resistance	Vertical	Outcrossing by honeybees	Brazil	Abud et al. (2007), Chiari et al. (2011)
	Conventional soybean	EPSPS	Glyphosate tolerance	Vertical	Pollen	Japan	Yoshimura et al. (2006)
	Glycine soja	EPSPS	Glyphosate tolerance	Diagonal	Pollen	Japan	Mizuguti et al. (2010)
Oilseed rape *Brassica napus*	*B. juncea, B. carinata*	EPSP	Glyphosate resistance	Diagonal	Pollen	Canada	Song et al. (2009), Seguin-Swartz et al. (2013)
	B. juncea	EPSP, bar	Herbicide resistance	Diagonal	Pollen	China	Song et al. (2010)

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	B. juncea	BtCry1Ac	Insect resistance	Diagonal	Pollen	Japan	Lei et al. (2011)
	Non-GM oilseed rape	Bar	Glufosinate resistance	Vertical	Pollen mediated	China	Cai et al. (2008)
	B. rapa *B. nigra*	CP4EPSPS, Cry1Ac	Insect and herbicide resistance	Diagonal	Glyphosate drift and selection pressure	USA	Londo et al. (2011)
Wheat *Triticum aestivum*	*Aegilops biuncialis*	Single major gene	Difenzoquat resistance	Diagonal	Hybridization	Spain	Loureiro et al. (2009)
	Aegilops cylindrica	Pch1	Disease resistance	Diagonal	Hybridization	USA	Perez- Jones et al. (2006)
	Aegilops cylindrica	ALS	Imidazolinone resistance	Diagonal	Pollen	North America	Gaines et al. (2008), Gandhi et al. (2006), Rehman et al. (2010)
	Dwarf male sterile line	Nib8	Wheat yellow mosaic virus resistance	Vertical	Pollination by wind	China	Dong et al. (2016)
	Non GM wheat	Bar and gfp	Herbicide resistance	Vertical	Pollen	Russia	Miroshnichenko et al. (2016)
Rice *Oryza sativa*	Weedy rice	ALS	Imidazolinone resistance	Vertical	Pollen	USA	Valverde (2013), Gealy (2005), Shivrain et al. (2009)
	Weedy rice	CpTI and Bt/CpT	Insect resistance	Vertical	Pollen	China	Cao et al. (2009)
	O. rufipogon	Bar	Herbicide resistance	Diagonal	Pollen	China	Wang et al. (2006)
	O. sativa F. spontanea	Bar	Herbicide resistance	Diagonal	Cross pollination	Spain	Serrat et al. (2013)

Genetically modified crops and gene flow

Cotton

Cotton is first genetically engineered crop which was commercially introduced in 1996. 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 refugees 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) 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. Cotton germplasm for the development of non-Bt strains in future.

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). The

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driving sources behind transgene flow are pollen transfer between hybrids of different transgene controlling certain traits, cultivator determined seed selection and mixing. But both traits are affected by farmer's practices and agroecological circumstances.

The cultivation of open-pollinated varieties (OPVs) along with commercial hybrids also increase rate of gene flow (Sanvido et al., 2008). 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). The role of seeds as an additional source for gene flow in maize must not be underestimated (Dyer et al., 2009). Farmers share and recycle maize seeds which increases gene flow locally but also increases the distance that transgene travel. Seed saving and sharing need to be analyzed along with PMGF to understand that what happens from local to regional, national and transnational levels, over time.

Soybean

Weeds are the major problem in soybean cultivation. To control plague a foreign gene CP4 was introduced to develop herbicide resistant soybean. Transgene flow becomes a major problem because the farmers prefer to grow conventional soybean. Cultivation of GM soybean has been increased many fold due to the introduction of glyphosate resistant soybean (Yoshimura et al., 2006). 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. Ray et al. (2003) reported 0.65 to 6.32% natural cross pollination in soybean in different experiments and highlighted the potential for transgene flow. Cross pollination in soybean is more likely facilitated by insects (Rust et al., 1980), since soybeans are predominantly self-pollinating and the flowers have anatomical features of entomophilous plant species (Erickson and Garment, 1979).

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. It is partially pollinated by honeybees and bumble bees and is also known to release large amounts of air-borne pollen. Timmons et al. (1995) demonstrated that cross-pollination in oilseed could vary from 5 to 55%. There are number of factors which control PMGF that includes flowering synchrony, mode of pollen dispersal (wind and insect), area density of donor, recipient plants and physical distance (Campbell, 1985).

Wheat

PMGF is main mode of transgene flow in wheat flower for disseminating transferred alien genes (Song et al., 2004). The gene flow in wheat usually occurs over very short distances and at extremely low frequencies, but measures should be taken to avoid contamination of non-GM wheat. In wheat, average cross-pollination rate is 1-2% in close proximity (Gustafson et al., 2005). Loureiro et al. (2007) demonstrated high rates of cross pollination (37 to 56%) by using emasculated wheat as pollen receptor at 0 m distance between two cultivars of *Triticum aestivum*. In another study, Loureiro et al. (2012) investigated that PMGF in transgenic wheat using three conventional wheat

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species and found a maximum outcrossing of 3.5%. Currently there are no commercial GM wheat varieties but extensive research is being carried out for the development of herbicide resistance either through genetic engineering or mutagenesis. Therefore, the concern is that once transgenic is commercially released there is potential for gene flow from GM to Non-GM wheats. So, the effective methods like isolation distances or containment and mitigation strategies should be opted for preventing outcrossing and contamination between compatible genotypes.

Rice

Rice is highly self-pollinated crop but pollen mediated outcrossing occurs when flowering period of various cultivars get synchronized and/or grown in close vicinity, but frequency of gene flow is very low (< 1.0%). Previous studies found that GM rice may hybridize with traditional cultivars (Rong et al., 2005; Yuan et al., 2007), weedy rice (Chen et al., 2004; Zhang et al., 2006; Olguin et al., 2009), and wild rice (Chen et al., 2004; Yao et al., 2008). Transgene flow from GM rice to other cultivars and weedy relatives is a major risk associated with commercial release (Messeguer, 2003). Development of GM rice varieties for having various traits like herbicide tolerance (bar and EPSPs), disease resistance (Xa21) and insect resistance (Bt and CpTI genes) are in pipeline for commercialization (Xia et al., 2011; Parisi et al., 2016). These developments warn the researchers to prioritize the studies related to risk associated with transgene flow from GM to non-GM rice.

Transgene containment and mitigation strategies

While dealing with trans-gene flow one should keep in view the situations according to transgenic crops. The research work on risk assessment during gene escape can be a useful aspect while countering gene flow (Chapman and Burke, 2006). In general, there are two approaches; either we can keep the gene in original GMO or can mitigate the effects (Gressel and Al-Ahmad, 2006). The possible containment and mitigation strategies are discussed here and their comparison on the basis of positive and negative aspects is given in *Table 2*.

Transgene containment strategies

Physical containment

Usually gene flow occurs through pollen or seed, so one way to contain transgene can be preventing seeds and pollen dispersal (Linder et al., 1998). This dispersal can be prevented using isolation of GM crop by using various physical barriers in addition to careful processing of seed (Arriola, 1997). Researchers have found effective solution by using pollen barriers, stopping insect flow in crops and physical isolation. Staniland et al. (2000) limited the out-crossing up to 0.015% complimenting the results of Morris et al. (1994) where out-crossing was reduced to 0.94% in two different experiments. All of this sounds very convincing when used in experiments but when we look at ground realities different shocking cases are reported like, scientists have found traces of trans-genes in seeds from non-experimental area. Landraces of maize (*Zea maize*) in Mexico was found with tarns-genes despite of fact that GM corn was not allowed to cultivate in the country (Ortiz-Garcia et al., 2005). Similarly glyphosate resistance was found in

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cultivars of *B. napus* grown in Canada, a year before cultivation of GM brassica was allowed to plant (Hall et al., 2000). A non-GM crop was found with trans-genes, this crop was cultivated (at long enough distance to reduce pollen contamination) after a GM crop carrying gene for a pharmaceutical product on a field of registered company following all the rules for isolation (Fox, 2003). More alarming situation will be faced when most of world crops will be transgenic and isolation will not be possible (Rieger et al., 2002). These situations can be addressed by more careful processing and transportation of seed from GM crops plants, isolation of cultivars having sophisticated genes with more sensitive markers.

Table 2. *Comparison of different transgene flow countermeasures for their positive and negative aspects*

Countering technique	Positive aspects	Doubts	References
Physical containments	• Easy and simple to use • Economical • Easily useable to all crops	• Not been able to contain transgene completely • It is almost impossible to stop flow through seed based products	Arriola (1997), Linder et al. (1998)
Biological/molecular containments			
Sterility	• Gain great results using complete sterility	• Male sterility fount to be leaky as it can serve as female parent • For complete sterility vegetative propagation is necessary so not possible in all crops • Farmer will not be able to produce own seed causing monopoly of seed companies	Daniell (2002), Schernthaner et al. (2003)
Clistogamy	• Biological control without use of any danger to gene pool	• Can cause inbreeding depression • Difficult to use in all crops • Some leakage has been observed	Husken et al. (2010), Gealy (2005)
Apomixes	• Good for fixing heterosis	• Difficult to attain • Can cause dispersal through pollen if not complemented by sterility	Bicknell and Kultunow (2004), Bhat et al. (2005)
Maternal transformation	• Can effectively hinders the dispersal through pollen • If complemented with female sterility it can be a good option	• Not possible in all crops due to biparental inheritance • Backcrossing of hybrid with GM crop can disperse the trait	Maliga (2004), Haider et al. (2009)
Incompatible genome	• No extra labor is required	• Can only possible in crops having multiple genomes • Compatibility with homologous genomes have been reported	Lu (2003)
Gene splitting	• It can be effective if complimented with other techniques	• Gene splitting alone can cause up to 25% gene flow in segregating generation	Dong et al. (2015), Wang et al. (2014)
Expression in virus	• Alone can contain transgene flow effectively	• Transgene will be good only for single generation	Kelloniemi et al. (2008)
GURTs	• Sound effective technique	• No evaluation yet • Issues regarding monopoly of seed companies	Swanson and Goschl (2000)
Transgenic mitigation	• It disable transgene irrespective of flow • Found good results in evaluating TM	• If transgene is not removed it can restore expression at any stage • Very minute quantity of transgene flow is still there it can slow down the process but cannot completely shut it down • Different blocking genes used can be a novel threat to biosafety	Gressel and Al-Ahmad (2006), Kuvshinov et al. (2001), Saurabh et al. (2014)

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Biological and molecular containment

This sort of containment utilizes genetic manipulation to create plants with less ability to disperse transgenes usually by interference in pollination and fertilization process (Moon et al., 2011). There are different processes which are implemented to contain transgene biologically. In the following sections it is tried to explain basic principle and examples of different mechanisms.

Sterility

Most of the times transgene flow occurs through pollens so implying the trait of male sterility can cordially reduce the problem (Daniell, 2002). This system is incorporated in cytoplasmic DNA and can be restored by environmental stimuli or restorer genes in nuclear DNA (Schnable and Wise, 1998). This technology is seemed to be less viable as it is reported that in field male sterile plants are not fully sterile. In addition, if male sterile plant is used as female parent it can recover transgenic trait as a result of backcrossing (Daniell, 2002).

Another technique seems more convincing and viable if compared with male sterility, it utilizes sterility of both the sex in plants and they can still produce viable seed (Seed sterility) this process imply the use of different deleterious genes which can only trigger under specific conditions/stimuli, i.e. temporal or site specific promoters (only express in gametes) or any chemical reaction (Ryffel, 2014). Schernthaner et al. (2003) proposed a technique to produce sterile seed; they used lethal genes (lethal for seed fertility) and these are closely linked with gene of interest along with repressing genes on homologous chromosome on same loci. After hybridization the repressor gene will segregate from combo of transgene and lethal gene will cause death of plant due to having trans-gene that will lead to counter trans-gene flow.

Cleistogamy

Cleistogamy is a modification of flower structure to promote self-pollination but it avoids outcrossing in barley, soybean and rice, and is effective mean against transgene flow (Husken et al., 2010). Cleistogamy can be induced by mutations or genetic engineering. In addition, various genes have been identified, i.e. OsMADS 2, OsMADS 1, OsMADS 3 and SUPERWOMAN 1 (SPW 1) in rice (Lee et al., 2003; Xiao et al., 2003; Prasad et al., 2005; Yadav et al., 2007), and Cly1 and Cly2 genes in barley (Wang et al., 2013). But most of these genes cause sterility due to interaction with reproductive parts of flower (Agarwal et al., 2007). Out-crossing was restricted to 2-6% by transformation of these genes but sterility was in question (Gealy, 2005). To restore the fertility, a mutant of SUPERWOMAN 1 named SPW 1^{145T} was discovered (Yoshida et al., 2007).

Apomixes

The use of apomixes is most successful method to stop trans-gene flow and this is also modification in floral structure that can be propagated by asexual means (Gressel, 2015; Kwit et al., 2011). Some of crops like banana, potato and sugarcane are naturally asexually propagated but introduction of this trait in other crop plants is tedious and time consuming work without effecting the seed production and this mechanism has also a property for fixing the hybrid vigor (Bicknell and Kultunow, 2004). Different

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techniques can be utilized for this purpose like apomixes, parthenogenesis and manipulating ploidy levels. As study goes on asexual reproduction it is taken that most easy and frequently considered way is using apomictic traits (Gressel, 2015; Ryffel, 2014). Apomictic plants produce seed without undergoing meiosis, but in combination with male sterility can be a more effective method for containment of unwanted gene flow (Bhat et al., 2005). According to an estimate about 400 plant species and 40 families reproduce through apomixes (Carman, 1997). So this trait can be used widely because 40 representative families are available as reference. The over-expression of various genes (*OsLEC1* and *OsLEC2)* enhances production of apomictic embryo. The gene namely *SERK* and *OsAPOSTART* are found responsible for apomixes induction in *P. pratensis* (Albertini et al., 2005). Use of apomixes as a containment strategy has been proven in GM-bahia grass where transgene flow was limited to only 0.2% (Sandhu et al., 2010).

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). This method can be useful in decreasing the frequency of transgene flow but cannot be effective in traits where complete leakage is found (Gressel and Al-Ahmad, 2006). As crossing is a two way process so GM crop can also serve as female parent so with backcrossing hybrids can recover the transgene and spread it (Stewart et al., 2003). An additional problem is that some species like brassica share cytoplasm along with nucleus during meiosis (Haider et al., 2009). Some research works revealed that there is 0.4% introgression of transgene occur while using this technique (Avni and Edelman, 1991). Another experiment showed that pollens transmit cytoplasmic traits in 3×10^{-4} hybrids out of 780000 (Wang et al., 2004). Ruf et al. (2007) showed in an experiment that transmission was 1.58×10^{-5}. This small leakage can be further controlled by complimenting this trait with female sterility.

Incompatible genome

One possible option against gene flow is to incorporate the transgene in such a way that its probability to disperse through gamete is at minimum value. Some crops like wheat contain different genomes (Werner et al., 1992). 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). This technique has some practical problems; firstly it does not work for all the crops, secondly there have been reports for partial compatibility of homologous genomes (Knott et al., 1989; Snow, 2002). This partial compatibility can play role for unintended gene flow.

Gene splitting

Hirata et al. (1990) discovered a genetic structure called as intein which are capable of protein *trans*-splicing, it can combine two different DNA sequences in shape of a single protein and splice out of mature protein. Dong et al. (2015) proposed a containment strategy taking intein as major working agents, they proposed to split the gene of interest into two different sequences and place far away in genome and combine them with inteins, as far away sequences have more chances to segregate during meiosis and named it as gene splitting. Wang et al. (2014) practically perform this phenomenon

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to produce GM tobacco. This technique showed no signs of transgenes into F_1 population but backcross population showed 25% plants with trans-gene.

Expression in virus

Another technique can be to use virus mediated expression of beneficial trait using shuttle vector. Successful expression has been observed using this technique (Kelloniemi et al., 2008). By this gene can be limited to virus and danger of dispersal will be minimum but problem with this technique is making transgenic every year will increase cost of seed production as GM will remain good for just a single generation (Gressel, 2015).

Genetic use restriction technology (GURT)

GURT or "terminator technology" is also one way to preventing transgene flow. This technology was developed by multinational companies to protect intellectual property rights (Swanson and Goschl, 2000). GURT can be categorize into variety based (V-GURT) and trait based (T-GURT) (Van-Acker et al., 2007). V-GURT works on principle of seed sterility while T-GURT works on limiting the exposure of a specific trait to other crops. V-GURT was found more effective as compared to T-GURT (Goeschl and Swanson, 2003). Lin et al. (2008) used RNAi mechanism to contain herbicide resistant gene in rice. RNAi gene was closely linked to glyphosate resistance gene (gene of interest) to avoid crossing over during meosis, the RNAi gene was used to silence a gene which confer resistance to another herbicide, bentazone so GM rice was resistant to glyphosate but susceptible to bentazone. By applying bentazone to following generation plants carrying escape genes were effectively abolished. Similar experiment was repeated by Liu et al. (2012) using double transgenic and found complimentary results. GURTS are more effective for transgenic with industrial products (Lee and Natesan, 2006). Major concerns about terminator technology are that it will create the monopoly of seed companies but despite that it is an effective technique (Hills et al., 2007).

Transgene mitigation strategies

Containment techniques pose a major question to counter with the problem as when they are assessed almost every technique allows a little leak (Gressel, 2015). Even a smallest leak cannot make sure the complete containment, it rather slow down the process or direct it to a single direction but ultimate results are similarly harmful even if it appears some time later, just the slightest chance to not spread trans-gene is if it have no selection advantage or unfit for spread (Ryffel, 2014). If somehow expression of transgene is limited or sequence is deleted from offspring/gamete it will be more efficient way to block unintended gene flow. Scientists have tried and still coming up with different methods to fulfill the goal, in general these techniques are termed as "transgenic mitigation (TM) techniques" (Ryffel, 2014). TM was found more efficient to deal with transgene flow for traits more beneficial to agriculture (Lee and Natesan, 2006). Gressel (1999) proposed a technique to decrease the fitness of volunteer plant by incorporation of a deleterious gene closely linked to transgene and causing negative selection pressure on volunteer plants. These genes can be for say breaking dormancy, reduce shattering and dwarfing genes. Some experiments were effectively carried out to

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show the effectiveness. Al-Ahmad et al. (2004) inserted herbicide resistance gene linked with a dwarfing gene into tobacco plant. Both genes were linked close to avoid later crossing over and confirmed by PCR. Results revealed that volunteer plants and hybrids had only 17% fitness leading fewer chances for them to survive. Using same model on B. *napus* revealed only 12% fitness in the following progeny (Al-Ahmad and Gressel, 2005). Efficiency of this technique is strictly depends upon level of unfitness of trait to volunteer plants and nature of GM crops, this system can be made more effective using two or more TM genes (Gressel and Al-Ahmad, 2006). Some effectively used TM genes are; giberallic acid insensitive conferring dwarfness, abscisic acid insensitive which break seed dormancy and SHATTERPROOF confer less seed shattering (Daniell, 2002). Kuvshinov et al. (2001) described a technique named as "recoverable block of function" (can also categorize under chemical TM). They incorporate a blocking sequence and a recovering sequence along with trans-gene, recovering sequence remains active in normal conditions and stops working under chemical control. Pollen excision also works on similar principals as it excises transgene from pollen or cause complete pollen sterility by triggering RNAi mechanism. Site specific mutagenesis or recombinase is utilized for this purpose (Saurabh et al., 2014). Moon et al. (2011) use a codon optimized serine resolvase recombinase (CinH) with its recognition sites along transgene, CinH was incorporated under influence of a pollen specific promoter LAT 59 to hinder the expression of transgene into pollen and ultimately hybrids. They found less than 1% pollens expressing transgene. In additions zinc finger nucleases, TALEN, CRISPR-Cas and Ecor1 restriction endoneucleases had also found effective to deletion of transgene from pollen (Straus and Lahaye, 2013).

Conclusion

Escape of transgene from GM crop plants to non-GM and wild relatives may pose potential environment risks. Understanding of transgene escape will facilitate the sustainable and safe cultivation of GM varieties of different crops. Further, perceived food safety and identity preservation is necessary for different production systems in agriculture sector and famers choice to cultivate GM, conventional or organic crops. 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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