Review

Transgene introgression in crop relatives: molecular evidence and mitigation strategies

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Incorporation of crop genes into wild and weedy relative populations (i.e. introgression) has long been of interest to ecologists and weed scientists. Potential negative outcomes that result from crop transgene introgression (e.g. extinction of native wild relative populations; invasive spread by wild or weedy hosts) have not been documented, and few examples of transgene introgression exist. However, molecular evidence of introgression from non-transgenic crops to their relatives continues to emerge, even for crops deemed low-risk candidates for transgene introgression. We posit that transgene introgression monitoring and mitigation strategies are warranted in cases in which transgenes are predicted to confer selective advantages and disadvantages to recipient hosts. The utility and consequences of such strategies are examined, and future directions provided.

Introduction

Populations of wild relatives (See glossary) and weedy relatives of crop plants that experience potential gene exchange have been the focus of research on transgene introgression. Indeed, the suggestion of potential outcomes of transgene introgression has provided the foundation for several recent scientific investigations in which events before introgression (e.g. pollination and hybridization) are subjects of interest [1–7]. A continued increase in research and acreage dedicated to transgenic crops [8,9] will likely lead to further examination of potential scenarios resulting from transgene introgression; the repercussions of which are often cast in the context of negative ecological effects (Figure 1). In one scenario, selective sweeps could lead to weed management problems in coexisting transgene-introgressed weedy populations [4,10], as well as introgressed wild or weedy populations that act as sources of invasive individuals [11] via dispersal (Figure 1a,b). The invasiveness herein described might be accentuated by rapid evolution in the introgressed population [12,13]. At another extreme, in a manner similar to that described previously [14–16], demographic swamping, coupled with selection against the transgenes, could lead to local extinction of native wild populations (Figure 1b). In addition, the introgression of transgenes

Glossary

Cleistogamy: Non-opening flower trait. Only self-fertilization is allowed within a plant because of the unopened flowers. Pollen that carries transgenic traits is not allowed to disperse and fertilize with other plants.
Cytoplasmic male sterility: Male sterility caused by gene expression in the cytoplasm. Cytoplasm is maternally inherited, therefore, the sterility along with transgenes integrated into the cytoplasm are maternally inherited.
Demographic swamping: The inundation of compatible propagules (e.g. pollen and seeds) experienced by a differentiated population. One relevant example entails copious pollen flow from large-scale agronomic fields, which results in a preponderance of hybridization in a small wild relative population.
Fitness: Relative number of offspring that contribute to future generations by one form compared with another [85].
Genic male sterility: Male sterility caused by nuclear gene expression. Generally, no pollen formation is expected as an outcome, thus, transgene escape and introgression via pollen can be avoided.
Introgression: The permanent incorporation of genes from one differentiated population into another compatible population via hybridization and backcrossing over multiple generations.
Invasiveness: Propensity to disperse away from a source population and establish new populations in new spatial locations. The usage of the term here is consistent with Elton's [86] original conception of the term for invasive species. Current usage of the term often implies that invasive species inflict negative environmental or economic effects in areas where they spread.
Linkage disequilibrium: Frequency of genetic alleles is more or less than the expected frequency because the alleles are not randomly associated at multiple loci. If transgenes could be integrated into the less frequent alleles, transgenes would not be expected to present in the progeny due to the loss of the alleles during the crossing process with other plants of different ploidy levels.
Ploidy: Number of chromosome pairs within the nucleus in a single cell. Anueuploidy refers to lack or surplus number of chromosome copies of the normal number.
Selective sweep: Rapid incorporation of genes from one differentiated population into another made possible by increased hybrid fitness.
Weediness: Characteristic traits of a weedy plant that permit it to thrive in human-disturbed habitat. These include: rapid early-season growth, high seed output, high seed dispersal, seed dormancy, and persistent seed banks, competitive with crop plants.
Weedy relative: Compatible relatives that spatially coexist with their crop relatives, or exist in nearby habitats that are also highly dependent upon human management activities.
Wild relative: Compatible relatives that exist in nonagronomic habitats that are not dependent on human management.

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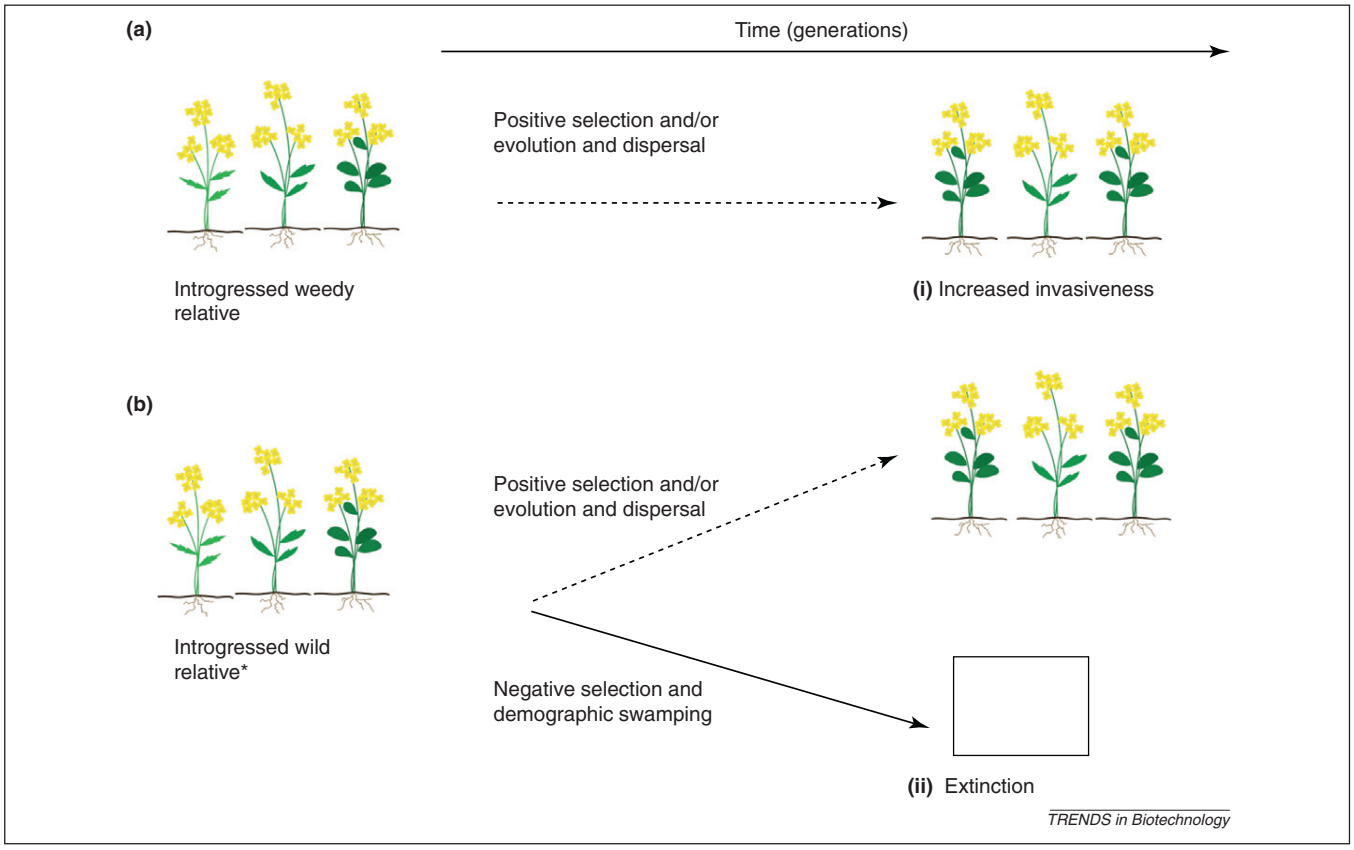

Figure 1. Two potential risks following transgene introgression from crops to their wild or weedy relatives. **(i)** Invasive hybrid population from introgressed (**a**) weedy or (**b**) wild population (dashed arrow representing seed dispersal) brought about by positive selection and/or evolution; **(ii)** extinction of wild relative population brought about by demographic swamping (e.g. copious pollen or seed dispersal from transgenic crops) and negative selection. We note that an introgressed coexisting weedy population might present problems for weed management when introgressed transgenes confer a selective advantage in the managed agronomic system (*), and that an introgressed wild population might also be of concern to managers of crop-wild relative genetic conservation (**).

into wild populations is of major concern in centers of diversity [17], because such a process could lead to gene pool modification, even in situations where selection on transgenic individuals is weak or nonexistent. Based on a lack of empirical evidence pertaining to the aforementioned potential negative outcomes, we posit that transgene escape and introgression generally have little or no negative environmental or evolutionary consequences, and only pose biosafety risks in specific cases; most notably when: (i) the associated traits confer novel or enhanced fitness or weediness, because increased weediness could necessitate new or increased weed control measures; and (ii) introgressed transgenes confer a selective disadvantage in small wild relative populations that exist in close proximity to the transgenic crop.

This review discusses recent molecular approaches to identify cases of introgression of crop alleles into wild and weedy relatives, and examines the few studies that have used molecular evidence to document actual transgene introgression, elaborating on why potential negative outcomes (Figure 1) have not been observed. We also review introgression management actions that target prerequisite conditions and transitions associated with the introgression process, and discuss the consequences of implementing such plans. Future research directions are proposed that might assist with more effective documentation of transgene introgression and formulation of containment strategies.

The process of introgression and its documentation

Introgression can be defined as the permanent incorporation of genes from one population into the genome of another reproductively integrated population through a series of crossing and backcrossing events [18]. With regard to crop plants and their wild and weedy relatives, others also stipulate that introgression does not include inadvertent and often genetically unstable chromosome transfer of genes [19]. Introgression requires a set of initial conditions, followed by multiple processes that occur through time and space (Box 1). In crop-to-wild and crop-to-weedy-relative systems, the introgression processes typically include hybridization and subsequent backcrossing events. If initial conditions are met, introgression might be possible; moreover, certain processes might be accentuated under variable initial conditions. For example, large crop populations (especially in the case of modern agronomic settings) can promote hybridization via copious pollen flow to individuals in smaller wild or weedy populations, especially if the related taxon is an obligate outcrosser, thereby making introgression more likely [16,20]. Failure at pre- and post-initial-hybridization steps could preclude introgression [21]. Indeed, initial conditions and subsequent demographic steps encompass the targets of transgene containment strategies (see 'Transgene introgression management' below). In cases in which initial conditions are met, monitoring and

documenting subsequent steps, especially those involving hybridization and backcrossing, are therefore necessary to attribute definitively to introgression the persistent presence of transgenic individuals in (formerly) wild or weedy populations.

The tactics used to document transgene introgression via molecular methods are usually different from those employed for non-transgenic crop-to-wild and crop-to-weed studies. A single gene is often the subject of interest in transgene introgression, therefore, rather than a set of molecular markers widely dispersed over multiple chromosomes, molecular methods that target diagnostic transgene sequences (or sequences of promoters or reporters), gene products, and their zygosity levels (homozygous versus heterozygous) are used to document initial steps (e.g. hybridization via transgenic pollen-mediated gene flow). Later, distinguishing between backcrossed transgenic progeny (i.e. actual introgression) and the presence of transgenic individuals that are caused solely by seed dispersal requires nuclear and cytoplasmic diagnostic molecular markers [22,23]. Undoubtedly, complementing such work with data on selection assays and phenotypic/morphological corroborations will assist in definitively identifying cases of transgene introgression.

Box 1. Process of introgression

The necessary initial conditions for the most common pathway for transgene introgression include:

- Transgenic agronomic populations (typically in designated fields, but possibly also in adjacent areas, such as roadsides) in close proximity with their wild or weedy relatives that exist in the nonagronomic matrix. Chromosome numbers need not be equivalent [6,35].
- Overlapping flowering phenologies: co-occurring periods of time when floral organs of species/populations are receptive to cross-pollination.

Given the conditions outlined above, the following events are necessary for introgression to take place (Figure I). This is the most commonly envisioned model of introgression, which involves an initial pollen movement step:

- Transgenic pollen flow to the wild or weedy relative.
- Fertilization (i.e. hybridization).
- Seed →seedling →adult transitions of the F1 hemizygous transgenic plant within the nonagronomic matrix where the wild or weedy relative population exists.
- Backcrossing of the F1 hemizygous transgenic plants with existing nontransgenic plants or transgenic agronomic plants, if present. (Note: continued backcrossing in the future is necessary; such events occur in the non-agronomic matrix if the transgenic crop is no longer present.)

Alternative pathway

The necessary initial conditions for an alternative pathway for transgene introgression to take place include:

- Transgenic agronomic populations (typically in designated fields, but also possibly in adjacent areas such as roadsides) in close proximity with their wild or weedy relatives, which exist in the non-agronomic matrix.

Given the condition above, the following events are necessary for introgression to take place (Figure II):

- Seed dispersal of transgenic plant into a wild or weedy relative population.
- Seed→seedling→adult transitions of the transgenic plants and wild or weedy relatives within the nonagronomic matrix where the wild or weedy relative population exists.
- Transgenic pollen flow to nontransgenic wild or weedy relatives.
- Fertilization.
- Seed-to-seedling-to-adult transitions of the F1 hemizygous transgenic plant within the nonagronomic matrix where the wild or weedy relative population exists.
- Backcrossing of the F1 hemizygous transgenic plants with existing nontransgenic plants or transgenic agronomic plants, if present. (Note: backcrossing events need to take place again; all such events occur in the nonagronomic matrix if the transgenic crop is no longer present.)

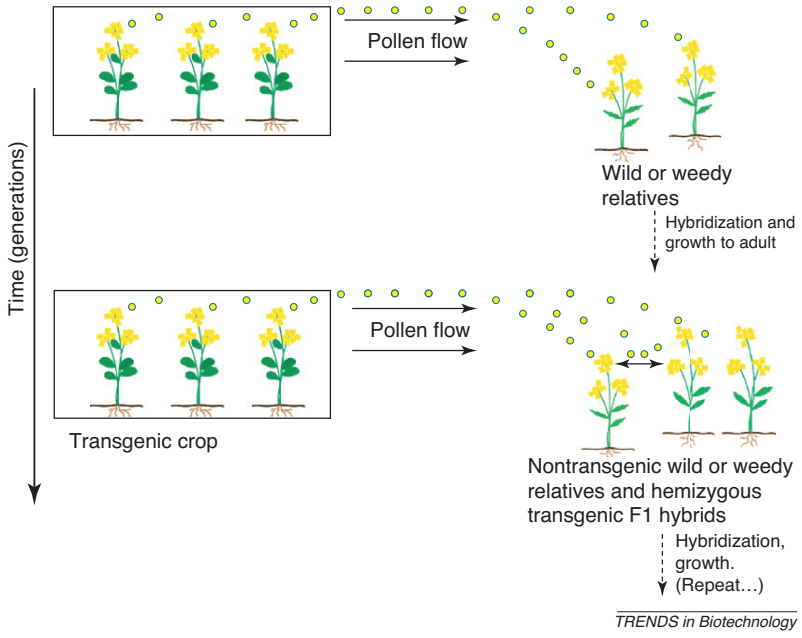

Figure I. Typically assumed pathway for introgression of crop alleles into wild or weedy relative populations, in which the initial step involves pollen flow from crops.

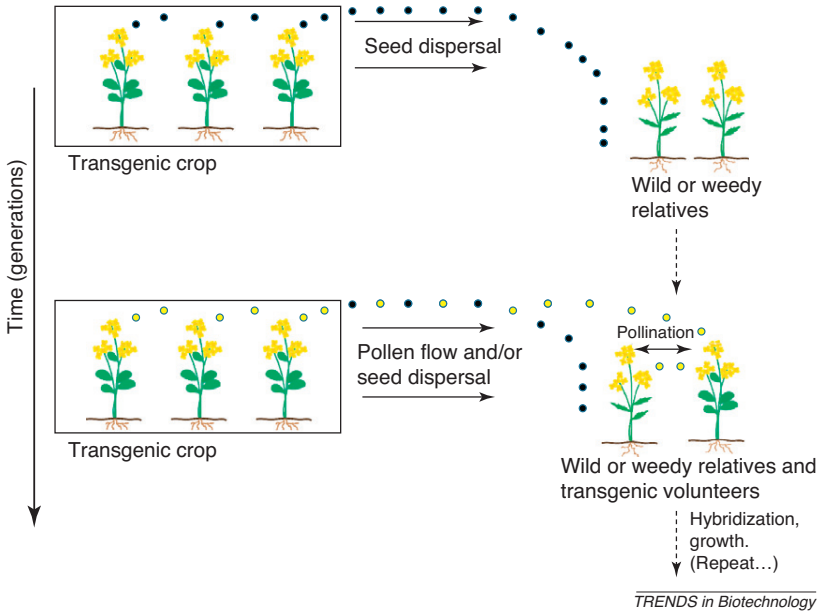

Figure II. Alternative pathway for introgression of crop alleles into wild or weedy relative populations, in which the initial step involves dispersal of crop seeds into the wild or weedy population.

In principle, the above scenario of introgression has played out in nontransgenic crops and their wild and weedy relatives [21]. Recent work in this area has emphasized the use of molecular markers to provide evidence of introgression (Table 1). Studies that address and suggest repercussions (e.g. fitness consequences) of introgression of nontransgenic crop genes in wild or weedy relative populations have been rare [24,25]. This may be because of assumptions that most domestication-associated genes confer decreased fitness in wild populations [21,26]. Inferences of introgression in such cases are typically made on the basis of single snapshots of population genetic structure and the inclusion of alleles that characterize, although not necessarily diagnostic of, crop populations into wild or weedy populations. Studies that have utilized Bayesian methods and admixture analyses have been useful in this approach, and have also provided evidence for ruling out cases of recent introgression [27–29]. However, long-term, multi-sample (i.e. longitudinal) efforts [30,31] have been utilized rarely to document the multi-step process of introgression. Despite their shortcomings, the highlighted studies in Table 1 have shed light on how selectively neutral transgenes could introgress into wild and weedy populations. It is also important to note that these examples of suggested introgression span the entire range of very low to high transgene introgression risk categories outlined in [21] (even those crops posited as very low risk [32,33]). Thus, although transgene introgression is likely to occur, automatic assumptions of negative outcomes should be tempered by the general lack of evidence of negative outcomes in cases of nontransgenic introgression.

Documentation of transgene introgression into wild or weedy populations has been even less substantiated. Arguably, the most exemplary study involved has quantified the presence of transgene-affiliated herbicide resistance and species-specific amplified fragment length polymorphism

Table 1. Recent (2005–2010) studies that provide molecular evidence of introgression from nontransgenic crops to their wild or weedy relatives

Crop	Relative	Molecular marker	Refs.
Cichorium intybus	*C. intybus*	AFLP	[30]
Glycine max	*Glycine soja*	SSR	[32]
Helianthus annuus var. *macrocarpus*	*Helianthus petiolaris*	RAPD	[87]
Medicago sativa	*M. sativa*	AFLP, SSR	[88]
Oryza sativa	*Oryza rufipogon*	SSR	[89]
Pennisetum glaucum	*P. glaucum*	SSR	[90]
Phaseolus vulgaris	*Ph. vulgaris*	AFLP	[33]
Raphanus sativus	*Raphanus raphanistrum*	Allozyme	[25]
Sorghum bicolor	*Sorghum halepense*	RFLP	[91]
Triticum aestivum	*Aegilops peregrine*	Fragment of noncoding locus	[92]
Vigna unguiculata	*V. unguiculata* ssp. *unguiculata* var. *spontanea*	RFLP	[93]
Vitis vinifera	*Vit. vinifera* ssp. *silvestris*	SSR	[24]
Zea mays	*Z. mays*	SSR	[94]

Abbreviations: RAPD, randomly amplified polymorphism; RFLP, restriction fragment length polymorphism; SSR, simple sequence repeat.

(AFLP) markers in transgenic *Brassica napus* (crop) × *Brassica rapa* (weed) hybrids (parents of different ploidy levels and chromosome numbers) that have backcrossed over several generations with weedy plants in field margins [31]. By the end of the study, immunological testing using glyphosate-resistance test strips had confirmed a 1:1 segregation of the transgene that conferred herbicide resistance in the offspring of an introgressed individual. Because this study of a natural weed population took place during a period of no herbicide-resistance selective pressure, it illustrates a situation in which a transgene – originally presumed to be neutral in the absence of herbicide use [34] – could introgress into a weed population. Similar results have been found in an experimental system (e.g. greenhouse and open-pollination) that involves transgene introgression from wheat (*Triticum aestivum* L.; $2n = 6x = 42$, AABBDD genome) into its weedy relative, jointed goatgrass (*Aegilops cylindrica* Host; $2n = 4x = 28$, DDCC genome), in which the *bar* gene, along with wheat-specific sequence-characterized amplified regions, was molecularly documented in the progeny of a selfed backcross generation [35]. Both examples profiled above involve interspecifc hybridization and introgression across a ploidy barrier, which brings into question the effectiveness of divergent ploidy levels as a mitigation strategy.

Rapid transgene spread has been documented recently for transgenic herbicide-resistant creeping bentgrass (*Agrostis stolonifera* L.) via sequence verification of the *CP4 EPSPS* transgene and analysis of nuclear internal transcribed spacer and chloroplast *matK* gene trees in nonagronomic habitats, only 2 years after a confined experimental trial released the transgene in the environment [22]. The authors have suggested that pollen-mediated intraspecific hybridizations, crop seed escape, and herbicide use all contributed to the spread of transgenic individuals into wild *Agrostis* populations. The final transgene introgression study that has shown molecular evidence is the recent re-evaluation of the presence of transgenes (more specifically, the widely used constitutive CaMV 35S promoter region of the transgene of interest) in Mexican landrace populations of maize (*Zea mays* L.) [36–38]. In this case, the presence of transgenes 3 years after the initial 2001 documentation [39] of hybridization suggests that introgression could have occurred. We note here that the two previous examples of crop transgene introgression (involving *A. stolonifera* and *Z. mays*), although molecularly documenting transgenic individuals and hybrids in wild and weedy populations, have not documented hybridization and backcrossing; hence, populations that are infiltrated by transgenic individuals via seed dispersal might be at the incipient stages of introgression (Box 1).

The most severe ecological outcomes of transgene introgression (Figure 1) have not been observed. To the best of our knowledge, well-documented introgression of a transgene that confers a selective advantage into a wild population has yet to occur, and spatially new populations of invasive transgenic plants (wild or weedy) that originate from transgene introgression have not been found. Thus far, only experimental work has indicated that such scenarios are possible [e.g. backcrossed herbivore-resistant transgenic sunflower (*Helianthus annuus*) progeny exhibit higher fecundity measures than nontransgenic sunflower in settings mimicking wild populations subject to herbivore pressure [40]]. Extinction of wild populations as the result of transgene introgression has not been documented. For weedy species, experiments have illustrated that herbicide-resistant and insecticide-resistant transgenic weed × crop hybrids can exhibit higher fitness than nontransgenic weeds in managed agronomic settings [4] and adjacent habitats with 'herbicide drift' effects [10]. Whether such weeds are more likely to become invasive and spread throughout the landscape has not been documented. Owing to the series of steps and conditions necessary to arrive at the most severe outcomes of transgene introgression, such scenarios are highly unlikely. Transgene introgression management plans might therefore be most appropriate for cases in which significant fitness advantages and disadvantages conferred by the transgenic trait are postulated for a recipient wild or weedy relative population.

Transgene introgression management or mitigation strategies

Management strategies aimed at preventing transgene introgression are intimately tied with challenging the initial prerequisite conditions and subsequent events (Figure 2). Most of the strategies developed target pre-hybridization steps (Figure 2a); far fewer target post-hybridization events (Figure 2b,c, Table I). In this section, transgene management strategies that have been integrated into transgenic crop plants are discussed, beginning with those whose actions serve to prevent hybridization, and concluding with those whose actions take effect post-hybridization. Potential negative consequences or pitfalls of the management strategies are also presented.

Male sterility

Pollen that carries a transgene is required in almost all transgene introgression models (Box 1). Hence, transgene introgression could be completely prevented if pollen does not develop (prevention of step shown in Figure 2a), and multiple methods have been used to decrease pollen fertility via genic male sterility or cytoplasmic male sterility (CMS). The first transgenic sterile male plant was generated by transforming tobacco (*Nicotiana tabacum* L.) plants with the chimeric ribonuclease *TA29* gene [41]. Since then, several efforts have been aimed at developing genic male sterility in plants. These include using cytotoxic *barnase* gene expression in pollen or anthers of poplar (*Populus*) trees and *Kalanchoe blossfeldiana* [42,43]. Plant-derived cysteine proteases and a gibberellin-insensitive (*gai*) gene have also been used to induce genic male sterility in *Arabidopsis* [44], whereas inactivation of the *UDP-glucose pyrophosphorylase 1* (*UGPase1*) gene for flower development has resulted in a genic male sterile phenotype in rice [45]. Such a strategy would be highly appropriate in crops whose primary economic purpose is not tied to successful fruit or seed development (e.g. biomass crops). Since genic male sterility strategies inhibit development of anther or pollen, the lack of pollen could create negative impacts on pollen-feeding insects [46].

Naturally occurring CMS, in which maternally inherited genes confer pollen-sterile plants, has been used

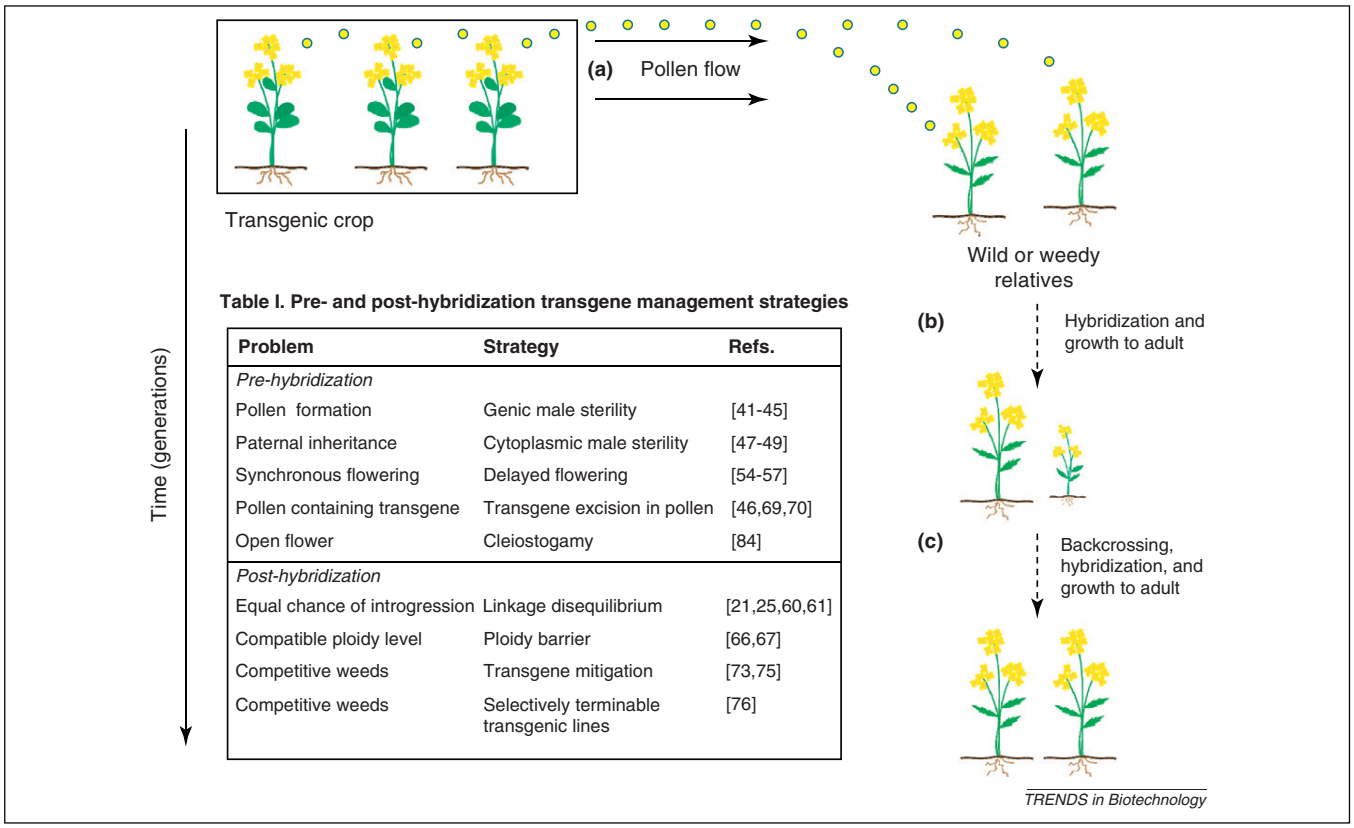

Table I. Pre- and post-hybridization transgene management strategies

Problem	Strategy	Refs.
Pre-hybridization		
Pollen formation	Genic male sterility	[41-45]
Paternal inheritance	Cytoplasmic male sterility	[47-49]
Synchronous flowering	Delayed flowering	[54-57]
Pollen containing transgene	Transgene excision in pollen	[46,69,70]
Open flower	Cleiostogamy	[84]
Post-hybridization		
Equal chance of introgression	Linkage disequilibrium	[21,25,60,61]
Compatible ploidy level	Ploidy barrier	[66,67]
Competitive weeds	Transgene mitigation	[73,75]
Competitive weeds	Selectively terminable transgenic lines	[76]

Figure 2. Stages along the portrayed transgene introgression pathway where transgene management strategies can operate. Pre-hybridization strategies are aimed at preventing cross-pollination, and typically target the transgenic crop (**a**). Post-hybridization strategies are aimed at decreasing the fitness of resulting transgenic hybrids (**b** and **c**). Pictured is the scenario of transgene mitigation, wherein a transgene-linked dwarfing gene results in shorter plants that are selected against in the wild/weedy environment. All mentioned strategies are incorporated into the transgenic crop.

heavily in plant breeding for hybrid seed production. More recently, genetically engineered CMS has been used in transgene biocontainment (prevention of hybridization step shown in Figure 2b). This started with the successful genetic engineering of the tobacco chloroplast genome with the *phaA* gene coding for β-ketothiolase, which is known to confer CMS [47]. Expression of an abnormal mitochondrial open reading frame *orf79* in rice has also resulted in CMS [48]. Disruption of a nuclear gene *Msh1* that is responsible for the suppression of mitochondrial DNA rearrangement has caused heritable CMS in tobacco (*N. tabacum* cv. Xanthi) and tomato (*Solanum lycopersicum* cvs. Moneymaker and Rutgers) [49].

A potential drawback of using CMS as a biocontainment tool is the potential for transmission of the transgene from the cytoplasm to the nucleus. Transmission of paternal plastids and mitochondria from transplastomic pollen occurs at low frequency (10^{-4} to 10^{-5}), and even less frequent transmission is expected under field conditions [50]. A lower than 10^{-3} transmission rate of chloroplast DNA through pollen has been suggested as an acceptable level for release of transgenic crop plants for large-scale cultivation [51]. Lack of stability of the CMS system under different environments might be an issue, but the CMS in maize, especially as conferred by T and C type cytoplasm, has been shown to be stable under various environmental conditions in three different countries over many years [52]. Moreover, pollen containment along with increased yield has been documented in maize systems that employ a combination of CMS hybrids and unrelated male-fertile maize plants [53].

Delayed and decreased flowering

Synchronous flowering time, at least partially, between transgenic crops and wild relatives is a prerequisite condition for hybridization. The development of a different flowering time of transgenic crops to avoid synchronous flowering with wild or weedy relatives would halt hybridization (prevention of hybridization step shown in Figure 2b), and a variety of gene-specific methodologies for shifting flowering time appears promising in this regard. Flowering time has been inhibited and delayed until vernalization treatment was applied by the overexpression of the *Flowering Locus C* (*FLC*) in *Arabidopsis* and *B. rapa* [54]. Flowering is significantly delayed by a repressor of floral development *TFL1* from grapevine in tobacco (*N. tabacum* L.) and *Arabidopsis* [55]. A putative CAATT-binding transcription factor has delayed flowering time in *Arabidopsis thaliana* under a long-day photoperiod [56]. *FLC* overexpression in *Arabidopsis* results in delayed flowering time and increased biomass [57]. Delays in or prevention of flowering might be tied to important biochemical pathways (e.g. ascorbic acid [58]), and furthermore, the genes linked to synthesis of enzymes in these pathways could prove useful in pre-hybridization management strategies.

A delayed flowering time strategy, particularly if accompanied by increased biomass, would be highly suited for

crops of primary interest in biomass production, such as switchgrass (*Panicum virgatum* L.) for biofuel and bioproducts. For agronomic crops cultivated primarily for seed production, significantly delayed flowering could result in less seed production because of an inadequate flowering period. Flowers in a single individual plant generally do not develop at the same time, therefore, complete flowering delay without overlapping time could require very significant delay for all flowers. The possibility of an overlap of flowering time could still exist between the earliest flower of a donor plant and the latest one of a recipient plant or vice versa.

Post-zygotic barriers to introgression

Even after successful hybridization between transgenic crops and wild or weedy relatives, undesired transgene introgression could be prevented if a transgene were inserted in a genomic region that would probably not be introgressed into the genome of pollen recipients, owing to linkage disequilibrium (LD). Transgene placement in loci that are located in crop genome regions that confer lower fitness and competitiveness to the wild relative are negatively selected and unlikely to be transferred to a wild relative under selection pressure (prevention of success of step shown in Figure 2c) [21]. Many studies on LD in higher plants have been conducted [59]. It has been demonstrated that certain crop-specific alleles can introgress easily and persist in wild relatives, whereas other alleles cannot [25]. Other studies have demonstrated preferred integration or insertion sites, including the existence of preferred DNA sequences for *Agrobacterium* T-DNA integration in *Arabidopsis* [60], and the observation of biased transgene insertion into specific maize chromosomes, using a site-specific recombination-system-containing vector [61]. However, LD does not only rely on the nature of T-DNA preference on integration sites. Advanced biotechnology allows targeted insertion of transgenes at pre-characterized loci [62]. Transgenes can be inserted at desired sites in a plant genome, because transgene insertion at a targeted locus is currently feasible using zinc-finger nuclease technology [63,64]. Indeed, site-specific integration of very large DNA fragments into any desired location has also been demonstrated by the expression of lambda-red enzyme in *Escherichia coli* [65]. However, target-site-specific/LD-linked transgene introgression management strategies might not be constantly reliable owing to frequently occurring recombination in the genome.

Different ploidy levels can suppress transgene introgression via pollen movement [66]. Intercrossing between individuals with different ploidy levels has resulted in dramatically reduced seed production with various phenotypic traits, including non-flowering hybrids in select grass species (prevention of success of steps shown in Figure 2b,c) [67]. Although inter-ploidy hybrids (e.g. triploids that result from crosses between diploid and tetraploid individuals) might be viable and contain the transgene, low hybrid fertility and aneuploidy that result from backcrossing could lead to a low probability of introgression of the transgene into the wild population. However, a transgene that confers herbicide resistance in hybrids between tetraploid *B. napus* and diploid *B. rapa* has been successfully introgressed in backcrossed hybrids over several generations [31]. Also, the use of ploidy barriers as a transgene introgression management strategy is limited to cases in which recombination is rare between different parental genomes [21]. Recombination and gene transfer, for example, have been shown to occur between A and C chromosomes in triploid hybrids of *B. napus* (AACC) and *B. rapa* (AA) [68].

Transgene excision and mitigation

Transgene introgression can be effectively suppressed before hybridization by removal of the transgene from the pollen (prevention of step shown in Figure 2b). Pollen-specific transgene excision using site-specific recombinases, such as Cre or FLP [69,70], is one method to create transgene-free pollen that carries only a noncoding recombination site. Efficient microspore-specific transgene excision has been demonstrated in tobacco (*N. tabacum* cv. Petit Havana SR1) using Cre recombinase directed by a microspore-specific NTM19 promoter [46]. Other recombinases, including ParA and PhiC31, that have been shown to excise transgenes in plants, have the potential to be used for pollen-specific transgene excision [71,72]. This transgene excision in pollen might be considered as a way of promoting future advances in terminator technology. This strategy allows continuous production of transgenic progeny seeds by using a pollen-specific promoter unlike the terminator technology that does not produce any seeds. Pollen does not carry any functional transgenes after transgene excision has occurred; therefore, only half of the produced seeds would be transgenic by the presence of the transgene in female gametes. This might be a pitfall of this transgene excision strategy. However, a possible way to produce homozygous transgenic seeds for commercialization has been suggested with incorporation of a conditionally expressed recombinase repression gene into the transgene excision strategy [70].

Alleviation of potential consequences of transgene introgression could be achieved by coupling a transgene with a mitigating gene, such as a dwarfing gene [73], even after transgene introgression has successfully occurred in the wild relative genome. The mitigating gene should have positive or neutral effects on crops (e.g. increased biomass or seed production of dwarf plants in crop systems [73]) and negative effects on weeds, because weeds would be rendered less competitive to compete for light (step in Figure 2c that results in selective elimination of transgenic individuals) [74]. Transgenic *B. rapa* × *B. napus* hybrids that contain a fitness-mitigating dwarfing gene have resulted in a significant decrease in the number of weedy progeny that persist through time under competitive conditions [75]. Such an approach could constitute a *post de facto* mitigation of a potentially adverse transgene introgression in a wild or weedy population. However, this transgene mitigation strategy would not be appropriate for gene transfer management from transgenic to non-transgenic crops growing in close proximity.

Selectively terminable transgenic lines

The creation of selectively terminable transgenic lines represents another strategy, as demonstrated in rice by

the tagging of a gene of interest with an RNAi cassette that suppresses the bentazon detoxification gene *CYP81A6* [76]. This has resulted in the creation of rice that is sensitive to a major herbicide, bentazon, to control weeds in a rice field [76]. Therefore, any possible hybrids outside of the field could be controlled by spraying bentazon during the conventional rice weed control process, even if transgene introgression were to occur in rice weedy relatives in or near the agronomic field (allowing selective removal of transgenic hybrids and allowing steps in Figure 2c to involve nontransgenic individuals).

Future directions

Documentation of the transgene introgression process

Research into transgenic crops is expected to increase dramatically, with the release of several new abiotic and biotic stress-tolerant transgenic crop lines and biofuel plant platforms. These traits will be the foci of future long-term monitoring programs because they have greater potential to alter plant fitness and to increase weedy or invasive tendencies [34,77], compared with traits in current commercial transgenic crops. Novel molecular strategies for monitoring and strategies for containment will also be foci of future studies. Monitoring approaches that survey transgenic crops and wild or weedy populations at crucial steps along the introgression process could also provide empirical data for enhancement, evaluation and utilization of population models [78–82] of transgene introgression.

Novel transgene introgression management approaches

Several management strategies currently show promise for further development. For example, cleistogamy (i.e. a condition in which flowers do not open and are instead self-pollinated in the bud) could be an effective strategy to prevent hybridization and transgene introgression. Increased utilization of cleistogamy is now possible in many agronomically valuable cereal crops by genetic engineering of class B floral homeotic genes [83]. Cleistogamous rice that harbors such a missense mutation in the class B MADS-box gene *SUPERWOMAN1* (*SPW1*) has been identified [83]. Other possible management strategies of transgene introgression that should continue to be explored include the potential use of the *Ph*1 gene or molecular chaperone acting gene from wheat, which is known to suppress recombination between homoeologous or homologous chromosomes. Prevention of transgene introgression into weedy relatives has been hypothesized using a transgene fused or linked with the wheat *Ph*1 gene [84].

It is clear from the empirical data reviewed in this article that many mitigation strategies, such as hybrid incompatibility and ploidy differences, earlier predicted to prevent transgene escape, are not individually foolproof. We should expect that even one in a thousand or one in a million probabilities will occur given the time and land area involved with agriculture. Therefore, it would be prudent to consider the incorporation of multiple biocontainment strategies within a transgenic crop. It will also be crucial to evaluate the potential consequences of escape of the containment strategy and the probable effect on wild or natural weedy relatives.

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