

Decreasing crops transgene persistence in volunteers and related weedy species: Oilseed rape (*Brassica napus*) case study

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Introduction

Some transgenic crops can introgress genes into other varieties of the crop, to related weeds or wild species, or themselves remain as “volunteer” weeds. Many of the engineered genes, such as those conferring resistance to herbicides, diseases, and to environmental stresses may possibly grant a fitness advantage to a weedy species growing in the same agricultural ecosystem, potentially enhancing the invasiveness or weediness of the resulting hybrid offspring that can compete with cultivated crops or natural populations (Stewart *et al.*, 2003; Gressel, 2005).

The addition of herbicide-resistance transgenic traits has been proposed as valuable for most of the economically important crops to control weedy relatives of the crops, but will become un-useful should the trait migrate to the weedy relatives. Gene transfer between transgenic and non-transgenic oilseed rape (*Brassica napus* = canola) varieties is well documented (e.g. Beckie *et al.*, 2003; Hall *et al.*, 2005). Oilseed rape cultivation traditionally results in significant volunteer populations during subsequent years because of extensive seed shatter. Volunteers can become feral as well as act as a genetic bridge for the transfer of transgenes to weedy relatives. Outcrossing of *B. napus* to weedy relatives is problematic because it is one of the few crops that has interfertile weeds occurring within the cultivated crop. Several studies have demonstrated the field transfer of genes between *B. napus* and the genetically close weedy *B. rapa* (wild turnip) (e.g. Warwick *et al.*, 2003; Hall *et al.*, 2005).

The proposed molecular mechanisms for transgene containment within crops (i.e. to prevent interbreeding, e.g. male sterility) severely restrict gene flow only in one direction, and allow low frequency of gene release (leakage). Gene flow (leakage) is inevitable even in that direction, allowing spread through the population of undesired species, unless mitigated (Gressel 1999; Daniell 2002). Thus, Transgenic Mitigation (TM) mechanism is needed to decrease the risky effects of transgene flow once it has occurred by lowering the fitness of recipients below that of the wild type competing species so that the transgene will not spread. In transgenic mitigation approach (Gressel and Al-Ahmad, 2009), a desired primary gene (e.g., herbicide resistance, pharmaceutical trait, etc.) is tandemly coupled with mitigating genes that are positive or neutral to the crop, but deleterious to hybrids and their progeny (e.g. dwarfing, non-shattering, anti-secondary dormancy genes, etc.). Dwarfism, which typically prevents lodging and increases crop yield while decreasing the plant ability to compete with tall plants in the wild (Gale & Youssefian, 1985), was used as a mitigator.

Materials and methods

A DNA construct of a dominant *ahas*^R (acetohydroxy acid synthase) gene from *Arabidopsis thaliana* conferring herbicide resistance, in tandem with the semi-dominant mitigator dwarfing Δ *gai* (gibberellic acid-insensitive) gene from *A. thaliana*, was transformed via *Agrobacterium tumefaciens* into oilseed rape (*Brassica napus*). The integration and the phenotypic stability of the tandemly-linked *ahas*^R and Δ *gai* genomic inserts in later generations were confirmed by PCR and Southern blot analyses.

Results

TM technology was efficient in the *B. napus* crop/*B. rapa* weed system in mitigating the risks of gene establishment in volunteers (Al-Ahmad *et al.*, 2006), and a related weed, as well as in the F₂ interspecific hybrid and backcross BC₁ progeny, without using the herbicide (Al-

Ahmad and Gressel, 2006). The dwarf TM transgenic *B. napus* had double the seed production when cultivated alone at typical crop spacing, but was exceedingly unfit in competition with non-transgenic cohorts in greenhouse and screen-house conditions, as well as exceedingly unfit when competing with wheat in an open field conditions (Rose *et al.*, 2009). At 2.5-cm spacing between the competing TM and non-transgenic plants, more than 90% of the TM *B. napus* remained stunted at the vegetative rosette stage and failed to flower. The TM *B. napus* reproductive fitness was zero at 2.5-cm and 4% at 5-cm spacing relative to the non-transgenic *B. napus* (Al-Ahmad *et al.*, 2006). At 3-cm spacing between the competing plants; both the non-transgenic and TM *B. napus* were more fit than competing *B. rapa*, while their hybrids with *B. rapa* were far less fit than *B. rapa*, and subsequent selfings and backcrosses were progressively less fit, especially those bearing the TM genes (Figure 1). The fitness of F₂ interspecific non-transgenic hybrids was between 50% and 80% of the competing weedy *B. rapa*, whereas the fitness of the comparable T₂ interspecific TM transgenic hybrids was never more than 2%. Finally, the reproductive fitness of the TM transgenic T₂ BC₁ mixed with *B. rapa* was further severely suppressed to 0.9% of that of the competing weed due to dwarfism (Al-Ahmad and Gressel, 2006; Figure 1). The linked unfit gene would be continuously manifested in future generations, keeping the transgene at a low frequency, and thus precluding its establishment and persistence in the agroecosystems.

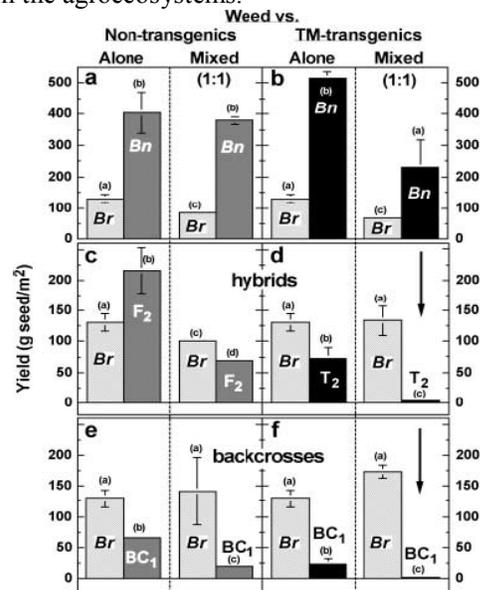


Figure 1. Strong suppression of the reproductive fitness of unfit transgenically mitigated (TM) *Brassica* genotypes when competing with weedy *B. rapa*. Br, *B. rapa*; Bn, *B. napus*; F₂ (non-transgenic) and T₂ (TM transgenic) selfed interspecific (*B. rapa* × *B. napus*) hybrids; BC₁, selfed backcrosses (*B. rapa* × F₁ or T₁ interspecific hybrids). Source: Al-Ahmad and Gressel, 2006.

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