Review

Plant Biotechnology, 16(1), 69–71 (1999)

Ecological Risks of Growing Genetically Modified Crops

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Genetic modification can become a major achievement to plant breeding. However, genetic modification differs from traditional breeding in that totally new traits—for example from unrelated organisms—can be added to plants at a high rate, and that these traits are usually introduced many at a time as precisely designed stacks of genes with their own regulating sequences. These differences demand that plants developed by genetic modification are risk assessed. The possible risks are that transgenic phenotypes with altered fitness could change in abundance in the ecosystem, with unwanted effects on other species and on ecosystem integrity or that the ecosystems are affected indirectly by the transgenic plants. The risk analysis should provide information about the following: (1) the possibility of transfer of the transgene by spontaneous crosses between crop and weedy or wild relatives, (2) fitness of the genetically modified crop as well as fitness of the crop relatives that received the transgene by introgression and (3) other types of transgene provoked interactions between the recipient plant and the environment. As an example of a risk analysis data are presented from the model genus *Brassica*.

1. Introduction

The risk of transgene transfer from oilseed rape (*Brassica napus*, 2n=38, genomic constitution AACC) to the weedy relative *Brassica rapa* (2n=20, genomes AA) has been studied. *B. rapa* is one of the parental species of oilseed rape. *B. rapa* is a common annual weed in agricultural fields worldwide in the temperate zone. Particulary in oilseed rape fields with a potential of introgression of oilseed rape genes from the crop to the weed. Outside the field *B. rapa* is ephemeral as seeds will only germinate when the soil is turned. Frequency of gene transfer as well as fitness analysis of offspring plants from crosses have been studied.

2. Results and Discussion

2.1 Frequencies of spontaneous hybridisation and backcrossing between oilseed rape and B. rapa

Our results indicate that spontaneous hybrids between oilseed rape and weedy *B. rapa* are easily obtained. The hybrids also backcrossed spontaneously to the weedy species in the field. The results have been reported [1–6] and they are summarized in **Table 1**.

B. rapa and interspecific transgenic hybrids were sown together in field experiments to assess the extent of backcrossing. Seed set per pod on interspecific hybrids was low (approximately 2.5) compared to seed set on the parental species (typically 16 to 23). The reciprocal cross *B. rapa* × hybrid did not seem to take place, as judged from analysis of seeds harvested on *B. rapa*. A large number of transgenic plants developed from seeds harvested on the interspecific hybrids, and individuals with a *B. rapa*-like morphology were selected for further analysis. Most of the selected plants were clearly backcross plants and a few were almost identical to *B. rapa* (chromosome number 2n=20, high pollen fertility) and had a high seed set in crosses with genuine *B. rapa* (**Fig. 1**) [6].

2.2 Analysis of marker transfer in controlled crosses and backcrosses between B. napus and B. rapa

Hybrids obtained from crosses between different maternal *B. napus* cultivars/transgenic lines and *B. rapa* individuals from cultivars or a wild Danish population were backcrossed (as females) to *B. rapa* individuals from the same population. The backcross, (*B. napus* cv. Drakkar × *B. rapa* cv. Indus) × *B. rapa* cv. Indus, has been analysed with RAPD markers to characterise the transfer of genetic material from the interspecific hybrid to the first backcross generation. We used 33 markers, specific to the *B. napus* parent of the cross. The vast majority of the markers were localized to the C-genome of the crop. All of the markers were transferred to the first backcross generation and most of them in a ratio that were not significantly different from 50% [7].

Another backcross progeny of the type (*B. napus* cv. Drakkar × *B. rapa* (weedy Danish population Bc 25)) × *B. rapa* (weedy Danish population Bc 25) was characterised with respect to both molecular markers, chromosome number and pollen fertility. Also in this cross all the markers—most of them specific to the C-genome—were found in the backcross plants. Plants with 20 chromosomes and a high pollen fertility, both characters of the weedy *B. rapa* were identified among the backcross plants.

2.3 Fitness of F_1, BC_1, F_2 and BC_3 plants from crosses between populations of B. rapa and varieties of B. napus (B. rapa as recurrent parent)

Plants from each of three weedy *B. rapa* populations and three *B. napus* varieties have been intercrossed, and

Table 1 Gene transfer from oilseed rape to *Brassica rapa* in spontaneous crosses

Hybridization, F_1	
female, *B. rapa*:	0–69% hybrids
female, *B. napus*:	0–9% hybrids
Backcrossing, BC_1	
female, F_1:	0–77% backcross plants (~1% *B. rapa*-like)
female, *B. rapa*	low

the fruit and seed set estimated. The offspring plants were grown in the field, and monitored for survival and reproduction. Combining these fitness components, hybrids were intermediate to *B. rapa* and *B. napus* (**Fig. 2**) [8].

Hybrids, *B. rapa* and *B. napus* plants, originating from crossings between two populations and two varieties in the first generation, were intercrossed to obtain F_2, backcross (on both *B. rapa* and hybrids), and pure *B. rapa* and *B. napus* seeds. The same fitness components as described above were estimated in the field for these offspring plants. In average, offspring from backcrossings and F_2 matings had a reduced fitness relative to offspring from intraspecific matings (pure species) [9].

A BC_3 generation produced from backcrossing *B. rapa* like BC_1 plants for another two generations were quantified as to their seed production and pollen fertility. With respect to these two fitness parameters there were no differences between the BC_3 plants and genuine *B. rapa*. This BC_3 generation segregated in a 1 : 1 proportion transgenic (herbicide tolerant) : nontransgenic plants. There were no significant differences between transgenic and nontransgenic sister-plants in survivorship or number of seeds per plant, indicating that costs associated with the transgene are likely to be negligible [10].

2.4 Isolating mechanisms reducing gene flow between B. napus and B. rapa

Differences in seed germination pattern between oilseed rape, wild *B. rapa* and their hybrids were investigated on seeds from controlled crosses (to get well characterised seeds of the two species and their reciprocal hybrids) as well as seeds from a number of natural populations of weedy *B. rapa*. As expected, all *B. napus* seeds germinated at 20°C and ambient humidity, showing no sign of dormancy. *B. rapa* on the contrary, showed a very high degree of dormancy. In this species we had to apply temperature cycling, KNO_3 and sometime even scarification to get a high frequency of germination. As to the hybrids, both types (*napus* × *rapa*) and (*rapa* × *napus*) seeds showed almost no sign of dormancy [11]. Lack of dormancy could be disadvantageous, as the number of hybrids reaching the flowering state could be limited due to germination under unfavorable conditions e.g. with effective weed management thereby limiting interspecific geneflow (**Fig. 3**).

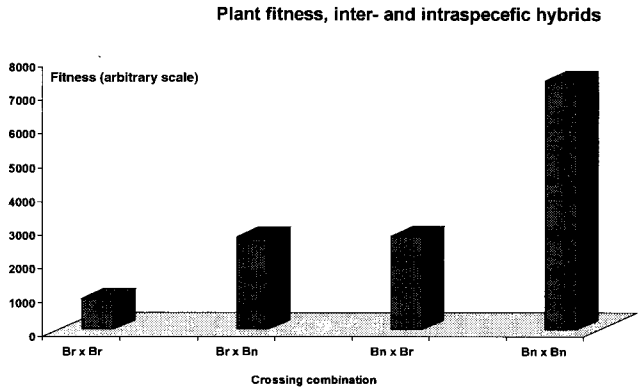

Fig. 2 Combined fitness estimate of F_1 hybrids and pure parental species (*B. rapa* and *B. napus*).

3. Conclusion

In conclusion we have found interspecific hybrid seeds in seed lots from both *B. rapa* and *B. napus*. Also adult hybrids and later generation introgression plants have been found to occur spontaneously in natural populations. The F_1, BC_1, F_2 and BC_3 generations are rather fertile and

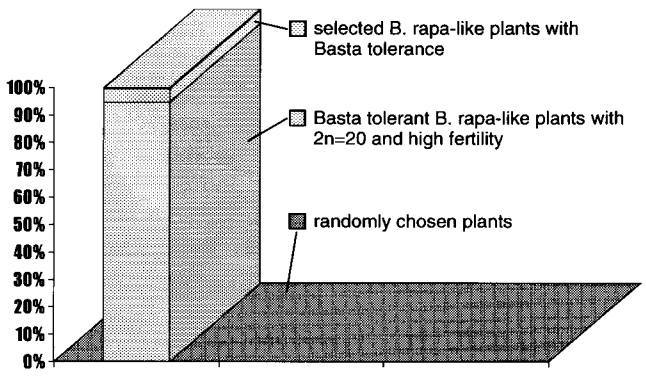

Fig. 1 The frequency of transgenic *Brassica rapa*-like plants in the first backcross generation between transgenic oilseed rape and *B. rapa* (*B. rapa* as recurrant parent).

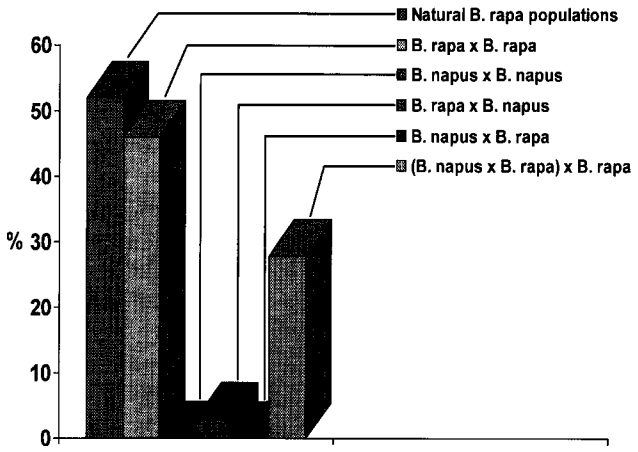

Fig. 3 Seed dormancy of *B. rapa*, *B. napus* and the interspecific hybrids.

fit and *B. rapa*-like plants were observed as early as the first backcross generation. Genomic positions providing "safe integration" of a transgene were not found, as all oilseed rape markers studied were transferred to the backcross plants from controlled crosses. Given these data it seems likely that transgenes will be incorporated into wild *B. rapa* populations. The consequences of this will depend on the transgene in question and the recipient ecosystem.

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