

Fitness and maternal effects in hybrids formed between transgenic oilseed rape (*Brassica napus* L.) and wild brown mustard [*B. juncea* (L.) Czern et Coss.] in the field

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Abstract

BACKGROUND: Gene flow between crops and weedy relatives depends on the survivorship and reproduction of early-generation hybrids in a field environment. The primary aim of this study was to compare the fitness of transgenic crop × wild hybrids with their parental types and a non-transgenic crop type in the field under enhanced temperature and humidity.

RESULTS: Transgenic insect-resistant oilseed rape (*Brassica napus* L.), wild brown mustard [*B. juncea* (L.) Czern et Coss.], their hybrids and non-transgenic *B. napus* were grown in such a way as to mimic field conditions after harvest under which volunteer plants might appear in agricultural settings. Factor analysis revealed that vegetative growth characteristics explained most of the observed differences among plant types. Wild brown mustard had the highest fitness during its entire life history. Hybrids had intermediate composite fitness and lowest reproductive fitness. The hybrid and the wild weed shared similar vegetative growth characteristics and seed dormancy in their respective progenies.

CONCLUSION: These data indicate that there might be enhanced persistence of the transgene in warmer climates. The absence of fitness cost of the transgenes might allow transgenes to persist in ecosystems. These data will contribute to risk assessments of transgene persistence and weed management against the backdrop of global climate change.

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Keywords: composite fitness; fitness cost; gene flow; global climate change; maternal effects; seed dormancy

1 INTRODUCTION

Introgression between transgenic crops and their wild relatives remains an area of concern because transgenes might increase fitness and competitiveness of wild relatives, increase their weediness and make them more difficult to control.^{1,2} Successful introgression depends on several criteria including sexual compatibility, proximal coexistence, overlapping flower times, production and fertility of F₁ hybrids and selective advantage. Furthermore, transgene location within the host genome and commensurate linkage drag are also important considerations.² Among these factors, of particular consequence is sexual compatibility, which affects the likelihood of hybridisation in the first place, whereas fitness of crop–wild hybrids influences the probability of sustained existence of transgenic hybrids. Although insect-resistant Bt *Brassica* crop plants have not yet been commercially released, Bt transgenic *Brassica napus* L. [oilseed rape (OSR)] and its wild relatives have been extensively studied as a model system, especially in risk assessment. Gene flow among several *Brassicacae* is well established. *Brassicacae* are also subject to insect damage.^{3,4} Most introgression studies between transgenic OSR and its wild relatives indicate that fitness of crop–wild hybrids is lower than that of the wild parental type,^{5–7} although some studies have demonstrated that fitness of hybrids is similar or even higher than that of their

wild parents.^{8,9} However, useful comparisons depend on various circumstances such as competition, plant density, environmental stresses and parental effects.^{7,9–13}

Both maternal and paternal effects can contribute to the growth and reproduction characteristics of hybrids and their progenies^{12,13} and thus affect the persistence and the following introgression of transgenes. Mercer *et al.*¹² found that the paternal effect (in this case, a domestication trait) in sunflower could increase the fitness of hybrid progeny from seed from the maternal wild parent. In contrast, seed dormancy of the reciprocal hybrids formed between wild and cultivated *B. rapa* L. more closely resembled their maternal parent.¹⁴ The maternal effect from the

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wild parent could increase the persistence of hybrid seeds in soil,¹⁵ thereby potentially increasing the persistence of transgenes in nature. Adaptive maternal effects would enhance performance of the offspring, since their environments would be similar to maternal environments under limited seed dispersal.¹⁶

There are various ways in which fitness of plants is estimated. Ecologists often estimate fitness by reproduction (e.g. seed production). However, it has been argued that competition in space rather than seed production limits the invasiveness of plants.¹⁷ In addition to reproduction growth, vegetative growth characteristics have also been used as fitness components, and traits involving entire life history have been used to estimate the fitness of plants in many studies.^{18–20} Hooftman *et al.*²¹ suggest that single fitness components alone would not have revealed the performance advantage of hybrids, and demographic vital rates, i.e. germination and survival, should be measured. In addition, the persistence and evolution of hybrids in nature depend not only on reproductive success but also on their establishment and competitive ability.^{15,22} Therefore, both plant growth and reproduction are important characteristics that should be taken into account as fitness components.¹⁸

Theoretically, transgenes conferring resistance are not always associated with advantages in nature; they might also have ecological or physiological costs. Fitness cost might reduce the reproduction and survival of the plant and might thus not support the persistence of transgenes in natural ecosystems. This could be an important consideration in the management of weeds carrying introgressed resistance from their transgenic crop relatives through gene flow. Fitness costs could be caused by pleiotropy, physiological costs of the resistance trait or effects of particular insertion sites within the genome.²³ Mostly, transgenic fitness costs have not been observed,^{7,24,25} but some studies have revealed fitness cost of an added resistance trait.^{17,23} Decreased fitness and competitive ability and resistance costs are thought to be reasons why no transgenic introgressed hybrids have been observed.^{2,7} However, the stable persistence of a transgene in the field was discovered in Canada, although at very low frequency (one plant),²⁶ which suggested an absence of fitness cost even in the absence of selection.

Compared with the relatively abundant research on the fitness of hybrids formed between transgenic OSR and the wild *B. rapa*, few publications address hybrid formation between OSR and wild brown mustard [*B. juncea* (L.) Czern et Coss.], a wild relative of OSR and a common weed on Chinese farmland that occurs sympatrically with OSR crops.²⁷ It has been reported that *B. juncea* is sexually compatible and forms fertile hybrids with OSR.^{3,6,28} Data indicate that gene flow between OSR and *B. juncea* could spontaneously occur in the field, but at a rate much lower than between OSR and *B. rapa*.²⁹ Fitness of F₁ hybrids between transgenic *B. napus* and *B. juncea* was reported to be lower in terms of pollen activity and seed production in a greenhouse experiment,⁶ but the field performance of hybrids is unknown.

OSR planted as a spring crop is typically harvested in early summer. Seed density of OSR from shattering is considerable at harvest.³⁰ These seeds can germinate to become volunteer plants after emergence in the field. OSR seedlings can emerge in the fall after harvest if soil moisture is adequate, but many of them die over the winter.³¹ However, farmers in northern China are likely to use agricultural films to protect plants from cold in moderately cold areas. OSR volunteers could survive and bloom in the following spring. Volunteer plants and feral populations might serve as important transgene reservoirs for

gene flow for volunteerism.² In addition, global climate change could cause increased temperatures, which, in turn, might cause increased persistence of volunteers over winter. These factors could potentially increase the risk of transgenic introgression to other OSR or wild relatives.³² It is reported that extensive transgene flow between OSR varieties has been detected in Canada, resulting in the generation of volunteers that contain three types of herbicide resistance.³³ If transgenes could confer increased survival, the enhanced persistence of volunteer OSR plants and hybrids in the field could increase pollen availability for unintended transgene flow. Thus, it follows that this adventitious presence might potentially be a factor for the evolution of resistance of insect pests.³⁴

Field experiments were conducted in this study during one artificial growing season through the winter in northern China, using agricultural films from autumn to spring to protect from frost damage. Growth and reproduction were compared among hybrids and their two parents (transgenic *B. napus* and wild brown mustard *B. juncea*) and non-transgenic *B. napus* 'Westar'. The purpose of this study was to mimic a field situation with increased temperature from global warming and to examine volunteerism of hybrids. Taking growth characteristics and reproductive traits into consideration, both the fitness of the hybrids and the fitness cost of transgenes in the field were investigated. This could aid in predicting the fate of transgenic hybrids in nature and contribute to current knowledge on risk assessment of transgene persistence and weed management against the backdrop of global climate change.

2 MATERIALS AND METHODS

2.1 Plants

Four plant types were utilised in this study: wild brown mustard (*B. juncea* var. *gracilis*, mustard), transgenic oilseed rape (*B. napus* cv. Westar, GT) and F₁ hybrids (hybrids formed between wild mustard (as female) × transgenic OSR; the non-transgenic OSR parent (Westar) was also used to reveal any fitness cost of the transgenes. Seeds of wild brown mustard were kindly provided by Prof. S Qiang of Nanjing Agricultural University (NAU), originating from a local field collection. *Brassica napus* 'Westar', a spring-type OSR, was transformed with the pSAM 12 plasmid containing genetically linked GFP and Bt *Cry1Ac* cassettes (GT) regulated by independent CaMV 35S promoters.³⁵ The third generation of the progenies of transgenic event 1 was used in this study. F₁ hybrids were produced by pollinating ten wild brown mustard plants of that local seed collection with pollen from more than ten transgenic OSR (GT) plants in a greenhouse.³⁶

2.2 Field trial

The field experiment was conducted in the experimental field of the Institute of Botany, Chinese Academy of Sciences (Xiangshan, Beijing, China, 39° 59' N, 116° 12' E) beginning on 3 November 2005. A total of 150 locations of plants were placed 45 cm apart and were assigned along each of four rows in a zigzag pattern to study plants in between the nearby rows within the field. The four types of seeds were assigned randomly among the locations. Each of the three types of *B. juncea*, Westar and GT occupied 30 locations, while F₁ hybrids occupied 60 locations. The seeds were randomly sowed in a hole at each location. The 3 cm deep and 5 cm wide hole permitted a random distribution of the three pure-line individually sown seeds at each location.

Experimental fields were covered with agricultural films (transparent polyethylene) from early November 2005 to the middle of March 2006, which enabled the survival of volunteers and the comparison of transgene-factor fitness under increased temperature and humidity.³⁷ Open pollination was allowed during the flowering period. Field management of the plants was performed identically to normal oilseed rape cultivation. Plants were harvested individually at maturity in June 2006. Seeds were dried in a sunny location under ambient temperatures for 24 h and were stored at a site with dry air and good ventilation. Chemical pesticides were applied to kill pest insects and to ensure a growth period free of pest insect pressure. Weeds were also controlled.

2.3 Measurements

Seedling emergence was recorded after removal of the agricultural film in the spring. At the beginning of April 2006, plants were culled to one plant per location following GFP fluorescence screening and transgene PCR analysis of F₁ hybrids.³⁶ The result of this assay was the retention of transgenic F₁ hybrids and removal of non-transgenic plants. Seedlings emerged at most locations for all four plant types. A total of 115 potential hybrid plants emerged from 53 of the 60 locations tested, among which 85 plants had Bt and GFP transgenes at 47 locations and nine plants at six locations were all false hybrids and thus removed. Finally, only 47 hybrids were retained at each of 47 locations, 25 plants at each of 25 locations for GT and Westar, respectively, and 28 plants at each of 28 locations for *B. juncea*. At harvest, the number of mature plants was recorded and seedling survival was calculated by the proportion of numbers of adult plants at harvest to the number of seedlings after culling at the early stage. The date of first flower opening was recorded for each plant to calculate the number of days from sowing to flowering [days after sowing (DAS)]. Plant height, diameter of plant canopy and above-ground dry biomass were measured at harvest for each plant.

Flower number of each plant was estimated by counting fruits and non-fruiting pedicels at harvest. At the same time, hundred-seed weight and total-seed weight per plant were obtained and were also used to calculate the number of seeds produced per plant. The fitness differential effect between species would be underestimated if the fitness consequences were evaluated only in terms of seed production alone without the effects of seed quality.³⁸ Thus, 100 seeds were randomly sampled from each of the four plant types with ten replicates for a germination test to check the seed quality produced by each of the four plant types. Seeds were placed in each of 40 petri dishes, with humidity maintained by occasionally adding appropriate volumes of distilled water. Dishes were kept at 26:23 °C day:night thermoperiods. Since germination did not occur 1 week after sowing, seeds were stored for 3 days at 4 °C to break seed dormancy, after which the germination experiment was continued. Germination rate was recorded and seedlings were removed daily from each petri dish. The experiment was conducted for 1 month. Dormant seeds were assumed to be those that did not germinate but seemed fresh at the end of the experiment, i.e. those that were not discoloured or mouldy, and had a firm turgid texture. Mouldy seeds were removed during the experiment, and the germination rate of seeds before (the first germination) and after the treatment at 4 °C (the second germination) were calculated.

2.4 Data analysis

MANOVA was performed using SPSS13.0 software.³⁹ Tests for significance were conducted for eight variables individually among

the four plant types, which included emergence rate, the number of days from sowing to flowering, plant height, canopy diameter, dry weight of above-ground biomass, the number of flowers, the number of seeds and total seed weight per plant. According to the homogeneity of variances, the means of each variant were tested either by Duncan's multiple range test or by Tamhane's multiple range test, as appropriate. The significant level was set at 0.05, and the corrected *P*-value was adjusted to 0.00625 for eight-variant MANOVA analysis after Bonferroni correction.

Moreover, factor analysis was conducted to group these eight measured variables and to estimate the fitness and fitness-related characteristics of plants. Although there are obvious biological relationships among the eight growth variables, the Kaiser–Meyer–Olkin (KMO) measure was calculated to test the sampling adequacy, and Bartlett's test of sphericity was conducted to check if the factor model was appropriate.⁴⁰ The weight sum of the scores of each principal factor (weight was the proportion of variance explained by each factor)⁴¹ was used to calculate the composite fitness.^{18,20} ANOVA was then used to study the difference in various fitness components among plant types.

3 RESULTS

3.1 Difference of each variable

Based on the corrected *P*-value of significant level, there were significant differences in the eight variables for the four plant types: $F_{3,112} = 18.964$, $P < 0.001$ for plant height; $F_{3,112} = 22.716$, $P < 0.001$ for canopy diameter; $F_{3,112} = 9.587$, $P < 0.001$ for dry weight of above-ground biomass; $F_{3,112} = 13.368$, $P < 0.001$ for number of flowers; $F_{3,112} = 46.744$, $P < 0.001$ for seeds per plant; $F_{3,112} = 35.247$, $P < 0.001$ for total seed weight. Variation between transgenic hybrids and their paternal parents was found during the whole life history except for the two prematurity stages. The emergence rate of *B. juncea* (mustard) seedlings (0.8 ± 0.06 , $n = 30$) was greater than that of F₁ hybrids (0.6 ± 0.04 , $n = 60$), and that of GT (0.7 ± 0.07 , $n = 30$) was intermediate between them, but the difference was not significant. No difference was found between the emergence rate of GT and that of the non-transgenic OSR (Westar) (0.6 ± 0.06 , $n = 30$). Similar patterns were observed in the number of days from sowing to flowering among these plant types, and no significant difference was found (Table 1). The seedling survival of F₁ hybrids (95.74%) was similar to that of *B. juncea* (96.43%). GT (88%) had a relatively lower seedling survival, lower even than Westar (92%).

The values of F₁ hybrids were similar to those of their maternal parent (mustard), but significantly greater than those of the paternal plant, transgenic OSR (GT), not only for characters associated with the vegetative growth characteristics at harvest ($P < 0.001$, Tamhane's test, plant height, canopy diameter and dry weight of above-ground biomass) but also for the flower number (hybrid versus GT, $P < 0.001$, mustard versus GT, $P = 0.003$, Tamhane's test). However, there were different trends for the other two variants related to reproductive growth. F₁ hybrids produced significantly fewer seeds than their maternal parent type (mustard) ($P < 0.001$, Tamhane's test) and their paternal parent genotype (GT), but the difference in the latter comparison was not significant. F₁ hybrids had a significantly lower total seed weight than their parents ($P < 0.001$, Tamhane's test) (Table 1). No differences were detected between GT and Westar for all these characters during the whole life history.

Table 1. Means (\pm SE) of the measured variants scored on the four plant types: non-transgenic oilseed rape (*Brassica napus*, Westar), transgenic oilseed rape (GT), wild brown mustard (*Brassica juncea*, mustard) and their transgenic hybrids (hybrids)^a

	Number of mature plants	Number of days from sowing to flowering (DAS)	Plant height (cm)	Canopy diameter (cm)	Above-ground biomass (g)	Number of flowers	Number of seeds	Total seed weight (g)
Mustard	27	163.0 (\pm 0.90) a	94.3 (\pm 3.76) a	83.7 (\pm 6.31) a	26.3 (\pm 3.98) a	1080.1 (\pm 163.63) a	13 303.9 (\pm 1893.10) a	13.6 (\pm 2.10) a
Hybrids	45	163.0 (\pm 0.87) a	100.9 (\pm 3.47) a	67.0 (\pm 5.06) a	18.6 (\pm 2.46) a	1235.7 (\pm 153.53) a	550.1 (\pm 119.69) c	0.5 (\pm 0.11) c
GT	22	159.1 (\pm 1.26) a	66.6 (\pm 3.83) b	30.7 (\pm 3.41) b	7.0 (\pm 2.52) b	250.3 (\pm 151.92) b	883.0 (\pm 124.16) bc	2.0 (\pm 0.25) b
Westar	22	159.6 (\pm 1.42) a	74.6 (\pm 2.94) b	31.6 (\pm 2.73) b	6.8 (\pm 0.93) b	126.1 (\pm 15.92) b	1398.9 (\pm 239.75) b	3.4 (\pm 0.51) b

^a Different letters indicate different values at $P = 0.00625$, which is a corrected P -value by Bonferroni correction as requested by MANOVA. Duncan's multiple range test was performed for days of vegetative growth, and Tamhane's multiple range test for others. DAS = days after sowing.

Table 2. Rotated component matrix^a by factor analysis for the composite fitness in four plant types: non-transgenic oilseed rape (*Brassica napus* 'Westar'), transgenic oilseed rape, wild brown mustard (*Brassica juncea*) and their transgenic hybrids (extraction method: principal component analysis; rotation method: varimax with Kaiser normalisation)

	Component		
	1	2	3
Emergence rate	0.208	0.207	0.691
Number of days from sowing to flowering	0.114	0.044	-0.854
Plant height	0.899	0.001	-0.054
Canopy diameter	0.840	0.393	0.107
Dry weight of above-ground biomass	0.857	0.434	0.105
Number of flowers	0.941	0.158	0.056
Number of seeds	0.190	0.964	0.107
Total seed weight	0.241	0.958	0.065

^a Rotation converged in four iterations.

3.2 Fitness and fitness-related characteristics

The KMO value for the factor analysis was 0.659, and Bartlett's test of sphericity showed a significant difference from identity ($\chi^2 = 1038.38$, $df = 28$, $P < 0.001$) of the correlation matrix. The present data satisfied these assumptions for the factor analysis, in which three common factors (principal components) were extracted from all eight variables. The first group of common factor components included plant height, diameter of plant canopy, above-ground dry biomass and number of flowers. The second group contained the number of seeds per plant and the total seed weight, which could be viewed as reproduction factors (Table 2). The third group comprised seedling emergence and the number of days from sowing to flowering. The first, second and third principal components could be assumed to be the vegetative growth factors at maturity, the reproductive growth factor and the prematurity growth factor respectively. These factors accounted for nearly 85% of the total variance of the principal component analysis model. The first, second and third factors accounted for 41.1%, 28.2% and 15.6% of the total variance of the principal component analysis model respectively.

At all of these three growth stages, the differences were significant among the four plant types ($F_{3,112} = 2.692$, $P = 0.05$ for prematurity growth characteristics; $F_{3,112} = 19.837$, $P < 0.001$ for vegetative growth characteristics at maturity; $F_{3,112} = 47.738$,

$P < 0.001$ for reproductive growth). GT was slightly greater than mustard and hybrids during the prematurity stage, while there was no significant difference between the two crops or between mustard and hybrids (Fig. 1A). Mustard and hybrids had higher levels of vegetative growth characteristics at maturity compared with GT and Westar, and GT had the same value as Westar (Fig. 1B). During the reproductive growth stage, mustard had the highest performance, whereas hybrids had the lowest value and GT was intermediate between their two parents. No significant difference was found between two crops (Fig. 1C). All multiple comparisons were conducted using Tamhane's test ($P < 0.001$).

ANOVA on the new synthetic values (composite fitness), which were formed by the weighted sum of the three factor scores, revealed significant differences among the four plant types ($F_{3,112} = 15.651$, $P < 0.001$). Mustard had the highest value of composite fitness based on the factor analysis (mustard versus hybrids, $P = 0.035$; mustard versus GT, $P < 0.001$; mustard versus Westar, $P < 0.001$, Tamhane's test) (Fig. 1D). F_1 hybrids were fitter than GT and Westar ($P < 0.01$, Tamhane's test). Westar and GT had similar values of composite fitness by factor analysis (Fig 1D).

3.3 Germination of seeds produced by various plant types

The seed used in the germination test was obtained from the four plant types. No significant differences in germination were detected among the progenies of the four plant types during the first 4 days, except for the lower value for hybrids compared with others on the second day ($F_{3,36} = 8.858$, $P < 0.01$). From the fifth day on, no seed germination was observed for *B. juncea* and F_1 hybrids, while all seeds of GT and Westar had already germinated. After exposure to low temperature (4°C) for 3 days, some seeds of mustard and hybrids germinated. There were significant differences in germination among the four plant types by the 20th day ($F_{3,36} = 24.253$, $P < 0.001$), and the germination rate of F_1 hybrids was significantly lower than that of mustard ($P = 0.005$, Tamhane's test). After 21 days, there was no seed germination (Fig. 2A).

At the end of the experiment, the germination rates were 99%, 95.5%, 87.9% and 67.8% for Westar, GT, mustard and hybrids respectively. Obviously, there was an overall difference among the four plant types in seed characters ($F_{3,36} = 75.499$, $P < 0.001$ for first germinated seed; $F_{3,36} = 43.828$, $P < 0.001$ for mouldy seeds; $F_{3,36} = 20.755$, $P < 0.001$ for dormant seeds; $F_{3,36} = 20.115$, $P < 0.001$ for second germinated seeds) (Fig. 2B). GT had more mouldy seeds and a slightly lower number of germinated seeds than Westar ($P < 0.01$, Tamhane's test), and thus it had a relatively poor

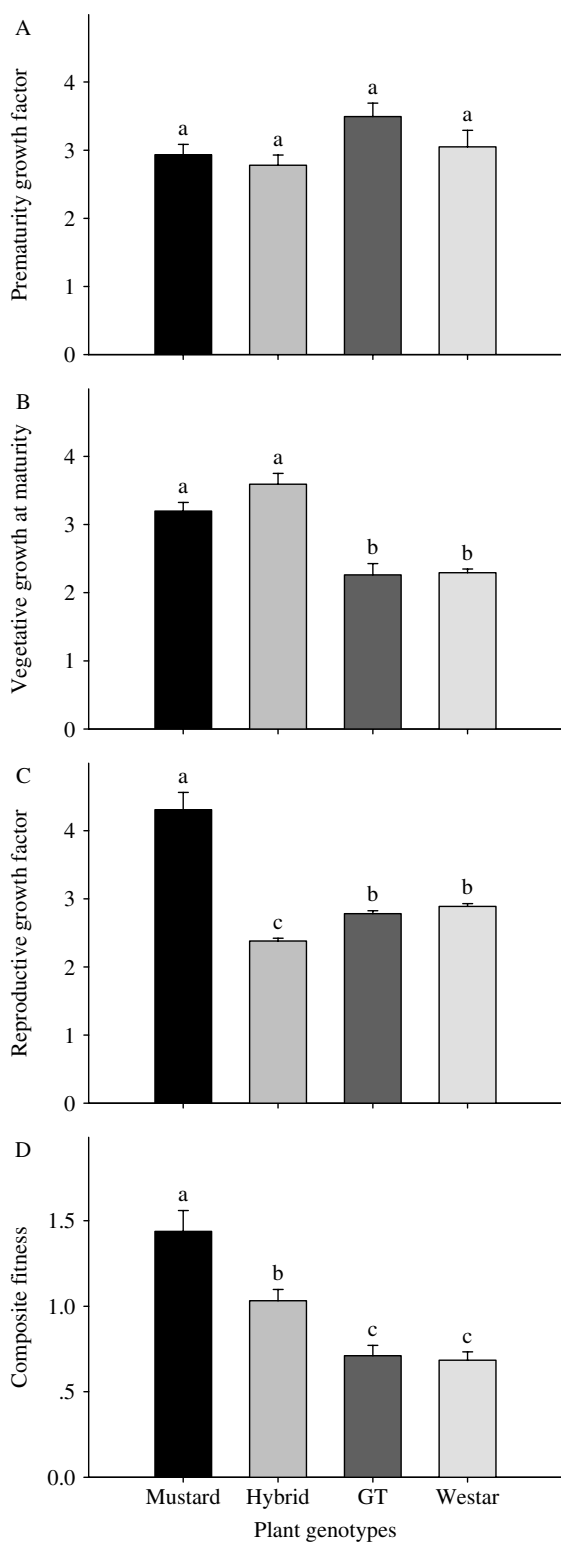


Figure 1. Plant growth and composite fitness by factor analysis for the four plant types – transgenic oilseed rape (*Brassica napus*, GT), wild brown mustard (*Brassica juncea*, mustard), their hybrids (hybrids) and the non-transgenic oilseed rape (Westar): (A) prematurity growth factor; (B) vegetative growth at maturity; (C) reproductive growth factor; (D) composite fitness. Error bars represent the standard error (SE) of mean. For GT, Westar, mustard and F₁ hybrids, 22, 22, 27 and 45 individual plants were calculated respectively. Different letters indicate significant differences at $P = 0.05$ (Duncan's multiple range test).

seed quality during the germination test compared with Westar. F₁ hybrids had the highest number of mouldy seeds and thus poorer seed quality than mustard ($P < 0.01$, Tamhane's test) and GT ($P = 0.001$, Tamhane's test), which resulted in decreased germination rates ($P < 0.001$ for hybrids versus mustard and for hybrids versus GT, Tamhane's test), although no difference was detected for the number of non-germinated seeds between F₁ hybrids and mustard. Dormancy was only observed in hybrids and mustard at the end of the experiment, and no significant differences were observed. There were fewer second germinated seeds for F₁ hybrids than for mustard ($P = 0.01$, Tamhane's test) (Fig. 2B).

4 DISCUSSION

4.1 Fitness of hybrids

The field environment of enhanced temperature and humidity conditions was more favourable to the growth of wild brown mustard, permitting a comparative analysis with transgenic hybrids under simulated global warming. Under global warming, patterns of temperature and humidity are subjected to change, thereby altering fitness landscapes, and comparative fitness among species and biotypes might change. The factor analysis revealed three common components, which were extracted from the eight original variables, and these had relatively important biological implications as well as statistical significance. According to the variables explained by common components, three growth stages were identified: the prematurity growth stage, vegetative growth at maturity and reproductive growth. These three categories were similar to the three groups proposed by Song *et al.*²⁰ based on related growth phases of plants. However, there was a slight difference in the classification of the life history stage compared with the earlier study, in which the number of flowers was artificially sorted into a reproduction factor,²⁰ whereas it was grouped into the vegetative growth category by factor analysis in the present study. Proliferative flowering could be related to vigorous vegetative growth as a result of heterosis, while low seed set might be the result of lower fecundity in hybrids. It is apparent that factor analysis is appropriate and useful here, as it could help reveal the underlying nature as it relates to fitness.

The reproduction of F₁ hybrids was lower than that of their parents, while the F₁ hybrids exhibited high rates of vegetative growth. The high competitive ability of hybrids during the vegetative growth stages could increase the persistence of transgenes in nature. It has been proposed that fitness assessments of transgenic hybrids should evaluate not only the reproductive traits but also the vegetative growth characteristics, as the establishment and competition ability of hybrids in nature could be the first criteria for survival, allowing transgene persistence outside the intended crop host. The number of flowers variable was included among the vegetative growth characteristics here, which presented pollen amounts provided by plants. A reciprocal backcross between transgenic hybrids and wild brown mustard demonstrates that the hybridisation compatibility was higher when the F₁ hybrids were used as pollen donors (unpublished data), which indicates a higher male fitness of the hybrids compared with female fitness. In other words, copious amounts of fertile pollen might compensate for the lower seed set of transgenic hybrids. Low seed set would not necessarily mean low risk of gene flow and transgene persistence in the field.

There was no transgene fitness cost observed in the present field experiment, which was consistent with previous reports.^{7,24,25} However, this could be due to the absence of stressful selection¹⁷

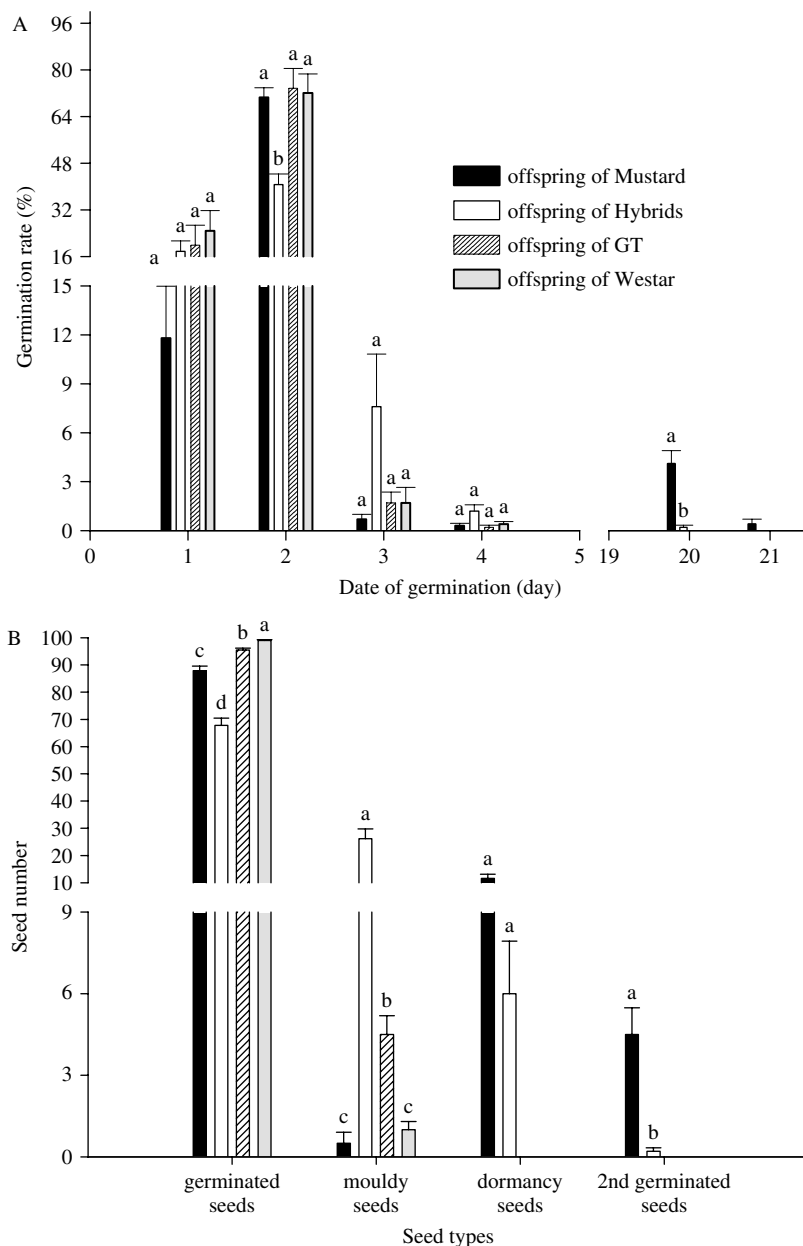


Figure 2. Seed daily germination rate (A) and seed characteristics (B) during the germination experiment for the four plant types: transgenic oilseed rape (*Brassica napus*, GT), wild brown mustard (*Brassica juncea*, mustard), their hybrids (hybrids) and the non-transgenic oilseed rape (Westar). Different letters indicate different values at $P = 0.05$ (Tamhane's multiple range test).

related to transgene products. Therefore, the performance of hybrids may be affected strongly by growing conditions, and further studies should investigate this. Transgenic plants with a neutral transgene would expect to volunteer at the same frequency as non-transgenic plants. In such cases, there might be negative effects in agriculture, such as more competition of transgenic weeds in crops, e.g. for light, nutrients, water and space, or influencing the economic income of farmers by reducing the purity of the crop seed.⁴²

In addition, density-dependent fitness comparisons have also been found in the hybrids formed between transgenic OSR and *B. rapa*, another common weed of *Brassicaceae*.^{7,9} In contrast, the present experiment was designed to mimic conditions without explicit competition to detect any fitness cost of transgenes in

the field. New experiments would be necessary to investigate the fitness of hybrids formed between transgenic OSR and wild brown mustard in various environmental conditions, e.g. in the presence of competition and stress.

4.2 Maternal effects

The composite fitness of F_1 hybrids, as determined by factor analysis, was intermediate compared with their two parents in this experiment. However, F_1 hybrids were similar to their weedy maternal parent in vegetative growth characteristics (Table 1). This suggests that maternal effects and heterosis in the hybrids might have a strong impact on vegetative growth under increased warming conditions favoured by the wild parent. In addition, seeds harvested from hybrids exhibited similar germination

characteristics to the wild parent. These results corresponded to other studies in which maternal effects were invoked.^{13,38} Maternal effects could affect some traits of seed, seedling and adult – nearly every stage of the life history. Seeds from GT and Westar, selected for low seed dormancy during domestication, had the highest germination rate, whereas dormancy of seeds from wild brown mustard is a typical weedy trait.³² Seed dormancy ensures that the weeds can survive in varying environments, while reduction in this trait can allow synchronous seed germination for effective cultivation of crops. Dormancy still existed in the second hybrid generation, a trait not found in OSR. The dormancy of hybrid progenies obtained from their maternal wild parent would facilitate the persistence of seeds in soil, which could enhance the risk of transgene introgression.

5 CONCLUSION

The high competitive ability of hybrids during the vegetative growth stages and the persistence of maternal effects could increase the persistence of transgenes in nature that are initially borne on transgenic crop pollen.¹⁵ It has been proposed that fitness assessments of transgenic hybrids should evaluate not only reproductive traits but also vegetative growth, as the establishment and competition ability of hybrid in nature could be the first criteria for survival. The frequency of transgenic hybrid production was low here, but fitness might be increased through further potential backcrosses with sympatric wild or weedy species in the field, as was shown in wild radish.⁴³ It was demonstrated that hybridisation between transgenic OSR and *B. juncea* could spontaneously occur,³ and that the backcrosses could result in better pollen fertility.⁴⁴ Gene flow from transgenic OSR to *B. juncea* populations should not be dismissed in risk assessments, especially in China and other sites where *B. juncea* is weedy in agricultural environments, since fertile hybrid progenies of similar fitness might be generated in further backcrosses with their wild parent. Maternal effects might increase the probability of the survival of hybrids in the field where temperature and humidity are increased and favoured by their wild parents under global warming scenarios. In addition, the similarity in dormancy between seeds from hybrids and those of the wild weed might provide an avenue for long persistence of transgenic seeds in nature without proper field management. In conclusion, there is the potential for gene transfer through gene flow between the two plants where the occurrence of wild brown mustard is close to the cultivation of transgenic oilseed rape, and transgenes might persist in nature, once transferred, where and when the survival of the hybrid is permitted at moderately warm temperatures.

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