POTENTIAL OF GENE TRANSFER AMONG OILSEED *BRASSICA* AND THEIR WEEDY RELATIVES

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INTRODUCTION

Transgenic plants of summer rape (*Brassica napus* L.) tolerant to the herbicide glyphosate have been tested in western Canada from 1988 through 1990. Prior to the commercialization of this tolerant material and the introduction of the herbicide tolerant gene(s) into *B. campestris* L. and *B. juncea* (L.) Coss. cultivars, the possibility of the glyphosate tolerant gene(s) being transferred through interspecific and intergeneric crosses among these cultivated species and into weedy relatives (*B. nigra* L. and *Sinapis arvensis* L.) requires investigation. Since *B. campestris* (AA, 2n=20), *B. napus* (AACC, 2n=38) and *B. juncea* (AABB, 2n=36) are related to *B. nigra* (BB, 2n=16) and *S. arvensis* (SS, 2n=18) (Mizushima, 1980), crosses between glyphosate tolerant cultivated forms and weedy species might occur. If successful, such crosses might produce glyphosate tolerant cruciferous weeds which would not be controlled with this herbicide. The objectives of this study were to determine the cross compatibilities among these species under both controlled and natural pollination conditions, and to assess the fertility of resulting interspecific and intergeneric hybrids and their selfed and backcross progenies in an attempt to estimate the potential for an escape of the glyphosate resistant gene into the weedy species.

MATERIALS AND METHODS

Six *B. napus*, one *B. campestris* and 13 *B. juncea* cultivars together with five *B. nigra* and four *S. arvensis* collections were used as parents.

Under controlled pollination conditions plants were grown in pots in the greenhouse or growth chamber under 16 h photoperiod and a temperature of approximately 20 °C. Buds which were about to open over the next two days were emasculated, immediately hand pollinated, and then covered with glassine bags for one week. The resulting seeds were harvested at maturity and planted in pots in the greenhouse. Hybrid plants were identified among the developing plants either morphologically or by their pollen viability, as determined by aceto-carmine staining of fresh pollen grains, or by root tip chromosome counting. Ovule culture was attempted in some crosses between cultivated and weedy species. Ovules were excised at 7, 9, 11, 13 and 15 days after pollination and cultured on M91 medium followed by a transfer to B5 medium. Hybrid plants were then transferred into pots and grown in the greenhouse.

To estimate the cross compatibilities among these species under natural field conditions, species were either grown in mixed stands or where individual plants of the female species were interspersed within plots of the male species. All seed set on the female plants was harvested. Hybrids were identified in three ways. First, harvested seeds were sown in pots in the greenhouse and the morphology and fertility of these plants were compared with those of parental species. Second, in crosses where glyphosate tolerant B. napus was the male parent, the harvested seeds were germinated on a medium containing 2.5-3.0 mM glyphosate. Seedlings tolerant to glyphosate were transferred into pots in the greenhouse and their hybrid status confirmed by examining their morphology and fertility. Finally, the remaining seeds were planted in the field at Saskatoon in 1990. Hybrid plants were identified in the field using a combination of morphological characteristics, fertility and plants maturity. Rooted cuttings of suspected hybrids were subject to cytological examination by meiotic chromosome observations of young buds.

The fertility of hybrid plants derived from controlled crosses was determined by allowing the plants to set seed without pollination assistance in the greenhouse, and also through selfing and backcrossing. The mitotic chromosome number of F_2 and backcross plants was determined in root tip cells. Root tips were immersed in 2.9×10^{-2} M 8-hydroxyquinoline for four hours followed by exposure to Carnoy fixative for a minimum of one hour. Root tips were then hydrolyzed in 1N hydrochloride at 60 °C for eight minutes. They were then stained with either Schiff's reagent, alum haemotoxylin or Carbol fuchsin. For meiotic chromosome observation young buds were fixed in Carnoy's solution for 24 hours and observed in acetocarmine squashes.

RESULTS

Interspecific and Intergeneric Cross Compatibility

Controlled crosses in the greenhouse and growth chamber conditions produced one or more hybrid seeds in 11 of the 17 interspecific and intergeneric crosses attempted (Table 1). Ovule culture yielded two hybrid plants, one from the cross B. napus x B. nigra and the other from B. napus x S. arvensis.

Under natural field pollination conditions at two sites over two years hybrids were identified from four of the 12 crossing blocks (Table 2). The natural interspecific crossing recorded was primarily among the oilseed Brassicas. However, five plants from the B. napus x B. nigra crossing block were classified as hybrids on the basis of their plant fertility, maturity and upper leaf attachment. Leaves on the upper stems of these five plants were sessile which is characteristic of B. nigra or B. juncea. However, when the metaphase cells of these five plants were examined, all were found to contain a number of bivalents in every cell. If they were true B. napus x B. nigra hybrids one would expect a chromosome count of 27 and very few, if any, bivalents. It was suspected that these plants might be the result of crosses with either B. campestris or B. juncea since these species were growing in neighboring crossing blocks. However the upper leaves of B. napus x B. campestris hybrids normally clasp the stem and all of the five plants had less than 37 chromosomes. Thus these five plants were not B. juncea crosses but whether they are the true hybrids of B. napus x B. nigra or the hybrids of B. napus x B. campestris is unclear.

Table 1. Controlled interspecific and intergeneric cross compatibility (CICC) of 17 crosses among four *Brassica* species and *S. arvensis*.

Cross		Buds poll.	Seeds prod.	Seeds plant.	Hybrids ident.	CICC [1] (%)
B. campestris	x *B. napus*	133	1733	120	1242 [2]	933.8
	x *B. nigra*	188	5	5	1	0.5
	x *S. arvensis*	86	5	5	0	0.0
B. juncea	x *B. napus*	1021	4434	228	4103 [2]	401.9
	x *B. nigra*	1419	98	98	42	3.0
	x *S. arvensis*	789	54	54	20	2.5
B. napus	x *B. campestris*	130	1989	155	1938 [2]	1490.5
	x *B. nigra*	742	103	103	7	0.9
	x *S. arvensis*	576	42	42	0	0.0
B. nigra	x *B. campestris*	130	0	0	0	0.0
	x *B. juncea*	1729	15	15	8	0.5
	x *B. napus*	973	7	7	1	0.1
	x *S. arvensis*	343	699	150	254 [2]	77.4
S. arvensis	x *B. campestris*	162	2	2	0	0.0
	x *B. juncea*	1067	26	26	0	0.0
	x *B. napus*	623	9	9	0	0.0
	x *B. nigra*	258	108	108	18	7.0

(1) CICC (%) = Number of hybrids developed x 100/Number of buds pollinated.
(2) Hybrids developed = Number of hybrids identified x Seeds produced/Number of seeds planted.

Table 2. Natural interspecific and intergeneric cross compatibility (NICC) of 12 crosses among four *Brassica* species and *S. arvensis*.

Cross		Plants observed	Hybrids identified	NICC [1] (%)
B. campestris	x *B. napus*	2129	21	0.99
B. juncea	x *B. campestris*	154	0	0
	x *B. napus*	6208	204	3.29
B. napus	x *B. campestris*	790	21	2.66
	x *B. juncea*	469	5	1.07
	x *B. nigra*	710	5 [2]	-
	x *S. arvensis*	745	0	0
B. nigra	x *B. campestris*	12	0	0
	x *B. juncea*	150	0	0
	x *B. napus*	1188	0	0
S. arvensis	x *B. juncea*	45	0	0
	x *B. napus*	7189	0	0

(1) NICC (%) = Number of hybrids identified x 100/Number of plants observed.
(2) The hybrid origin of these plants was not clearly identified.

Fertility of F_1 Hybrid Plants

Pollen viability of hybrid plants was reduced and varied among the different crosses (Table 3). Under open pollination, hybrid plants between the oilseed Brassicas produced a small amount of seed (data not shown) while the hybrids between the oilseed Brassicas and the weedy species produced few if any seeds. On backcrossing the number of seeds set was greater when the higher chromosome species was used as the recurrent parent (Table 3).

Fertility and Chromosome Number of F_2 and Backcross Plants. B. campestris x B. nigra: F_2 plants had near normal pollen viability but produced no seed on open pollination. Backcrosses of F_1s to B. campestris produced 323 seeds but only 28 plants. Ten of the 28 plants were grown to maturity and only one plant set six seeds under open pollination. The reciprocal backcross resulted in 31 seeds that grew into 11 plants. Open pollination of these plants produced only a few shrunken seeds.

B. juncea x B. nigra: Open pollination of F_1 plants produced 24 seeds which grew into nine plants. These plants had about 60% viable pollen and produced a few seeds on open pollination. The chromosome number (2n) of three of the nine plants were 25, 27 and 37. Ten F_1 x B. juncea seeds developed into five plants which under open pollination produced a few seeds. Two plants were examined cytologically and had 26 and 31 chromosomes (2n).

B. nigra x B. juncea: Twenty three seeds from F_1 x B. juncea backross grew into 13 plants with PVs varying from 23-95%. Most of these plants produced no seeds under open pollination.

B. juncea x S. arvensis: Seed produced on backcross F_1 x B. juncea failed to germinate and the one seed from F_1 x S. arvensis developed into a weak, male sterile plant which produced no seed on open pollination.

B. napus x B. nigra: Ten open pollinated seeds from F_1 plants grew into four plants which had 0-3% PV and yielded 29 seeds on open pollination. Three of the four plants had 38, 41

Table 3. Pollen viability (PV) and seed set of F_1 interspecific and intergeneric hybrids upon open pollination, selfing and backcrossing.

Hybrids derived from cross P1 x P2		PV[1] (%)	Seed set/Flowers pollinated: Open poll.	Self.	F_1 x P1	F_1 x P2
B. camp.	x B. nigra	28	52/25	0/18	323/80	31/53
B. juncea	x B. nigra	13	24/-	-	10/944	0/1821
B. nigra	x B. juncea	7	0/242	0/419	0/933	23/1361
B. juncea	x S. arvensis	16	0/790	0/54	1/1003	1/881
B. napus	x B. nigra	1	10/-	-	6/936	0/1274
B. nigra	x B. napus	3	0/-	-	1/308	5/313
B. napus	x S. arvensis	-	0/-	3/474	25/402	0/190

(1) PV (%) = Number of stainable pollen grains x 100/number of pollen grains observed.

and 45 chromosomes (2n). Six seeds from the backcross F_1 x B. napus developed into three plants which had 14-41% PV and set a few seeds on open pollination. Two of the three plants had 36 and 46 chromosomes (2n).

B. nigra x B. napus: Seed obtained from the backcross F_1 x B. nigra failed to germinate while five seeds from F_1 x B. napus backcross produced one plant with a 2n chromosome number of 27. This plant was male sterile and yielded two seeds on open pollination.

B. napus x S. arvensis: Two of the three F_2 seeds developed into plants which had 0 and 8% PV. One plant produced one seed on open pollination and the other plant was sterile. The chromosome number (2n) of these two plants was 35 and 36, respectively. Twenty three plants developed from 25 seeds obtained from backcross F_1 x B. napus. These plants had 0-23% PV and set 27 seeds on open pollination. Seven of the 23 plants had 34, 36, 36, 36, 46, 46 and 47 chromosomes (2n).

DISCUSSION

Under natural pollination conditions in field crossing blocks frequent interspecific crossing occurred among the oilseed Brassicas, B. napus, B. campestris and B. juncea. Since these cultivated species often grow in the same geographical area and may overlap in flowering periods, natural crossing among these species does and will continue to occur. Since B. napus has the A-genome in common with the other two species and this genome retains traces of homology with the B and C genomes (Mizushima, 1980), crossover between the chromosomes of these genomes could take place in the hybrid. In addition the production of a small amount of viable seeds on the hybrid plants under open pollination indicated that these hybrids could survive to the next generations. Thus gene transfer from B. napus to B. juncea and B. campestris in nature is possible. However B. campestris plants usually flower one to two weeks earlier than B. napus and crosses between these two species were more successful when B. campestris was used as the male. Therefore crosses between these two species will be less frequent than what have been observed in this study. In addition the progeny would quickly revert to B. napus unless the hybrid is backcrossed to B. campestris (Salam and Downey, 1978), a much less likely event than the reciprocal backcross. Thus the possibility of gene transfer from B. napus to B. campestris through natural pollination under field conditions is greatly reduced.

Although crosses between B. nigra and B. napus, and between B. nigra and B. juncea yielded a few hybrids, gene flow between B. nigra and these two species is greatly reduced by the low success rate of these crosses and sterility of the hybrid plants. The fact that no hybrid plant was found in the cross B. nigra x B. napus resulting from natural pollination among more than 1000 progeny plants investigated suggests that natural crossing between these two species is very remote when B. nigra is the female. Since the hybrids obtained were more easily backcrossed to B. napus and B. juncea than to B. nigra, the offspring of such an interspecific crosses would quickly revert to the cultivated amphidiploid species. This observation was supported by high chromosome numbers of all the hybrid progenies. Thus gene transfer from B. nigra to B. napus and

B. juncea by controlled crosses and backcrosses may be possible while the gene transfer in the opposite direction is very difficult even under controlled pollination. Thus the accidental escape of a glyphosate resistance gene(s) to B. nigra is, therefore, extremely unlike. Since B. nigra is not adapted to western Canada such gene escape would not occur in this major Brassica oilseed producing region.

The cross between B. campestris and B. nigra was successful only when B. campestris was used as the female. This indicates that the possibility of gene flow from B. nigra to B. campestris is greater than the reverse flow. The fact that it was much easier to backcross the F_1 plant to B. campestris than B. nigra further supports this conclusion. Since under controlled crosses about 2000 pollinations were necessary to produce one hybrid seed when B. campestris was used as the female, the possibility of this natural interspecific cross would be zero to extremely low. Thus even though a B. campestris cultivar tolerant to glyphosate was produced and commercially grown, natural gene transfer from B. campestris to B. nigra in western Canada would be zero.

The failure of seed set in reciprocal crosses between B. campestris and S. arvensis indicates that gene flow between these two species is extremely remote even under controlled crosses. The crosses between S. arvensis and B. napus and between S. arvensis and B. juncea were successful but only when S. arvensis was used as the male, indicating that gene transfer from B. napus and B. juncea to S. arvensis was very difficult even with controlled crosses. The high hybrid sterility under open pollination conditions greatly limites the possibility of any gene transfer to S. arvensis unless forced selfing or backcrossing is employed. Even then, the recovered progeny would revert to B. napus and B. juncea like plants since all F_2 progeny examined had chromosome numbers approaching their B. napus or B. juncea parents. In addition the F_1's were much more easily backcrossed to their amphidiplioid parents than to S. arvensis. Even though more than 7000 progeny plants were observed in the cross S. arvensis x B. napus no hybrid plants resulting from natural pollination was obtained, clearly demonstrating that this cross is extremely remote under natural pollination. Therefore, gene flow from oilseed Brassicas to S. arvensis by natural pollination under field conditions is not a hazard.

It is concluded from this controlled and natural crossing experiments that although gene transfer among the oilseed Brassicas under natural conditions can and probably does occur the natural barriers for such gene to flow to the weedy species, B. nigra and S. arvensis, is formidable and would not occur.

REFERENCES

MIZUSHIMA, U. 1980. Genome analysis in Brassica and allied genera. In: Brassica crops and wild allies. S. Tsunoda, K. Hinata and C. Gomez-Campo (eds.). Japan Sci. Soc. Press, Tokyo. pp. 89-106.

SALAM, M.A. and DOWNEY, R.K. 1978. Results of interspecific crosses between Brassica napus L. and B. campestris L. Bangladesh J. Scientific and Industrial Research 13: 1-4.