GENETIC TRANSFER AMONG CULTIVATED POLYPLOID BRASSICA SPECIES

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INTRODUCTION

The genetic transfer between three cultivated polyploid species of Brassica, B. napus ($2n=4x=38$, AACC), B. juncea ($2n=4x=36$, AABB) and B. carinata ($2n=4x=36$, BBCC) is of great interest. These species have developed in different areas and under different agroclimatic conditions. Each one was originated from two diploid species: B. nigra, B. oleracea or B. campestris. B. napus and B. juncea have been improving by modern breeding procedures for several decades while B. carinata started to arouse interest in the eighties. This species is important in its own right as a crop suitable for winter sowing in warm Mediterranean-type climates and for spring sowing in arid areas (Fereres et al., 1983). In turn it may be a donor of genetical traits such as resistance to drought, several diseases, and other characteristics such as non-shattering pods and yellow-coloured grains (Gálvez Ramírez and Romero Muñoz, 1984; Sacristán and Gerdemann, 1986; Sjödin, 1989).

In this paper we have analysed the different routes of genetical transfer among cultivated polyploid Brassica species with a view to improving B. carinata. Cytological information could permit the everyday use of these routes in classic breeding programs, without requiring the chromosomes counts and meiotic analysis.

MATERIALS AND METHODS

In this research program the following germplasm was used: two low erucic B. juncea cultivars (BJ) sent to us by Dr. Oram from CSIRO, Australia; eight double low Koipesol, S.A. spring B. napus cultivars (BN) and five Koipesol, S.A. B. carinata ecotypes (BR) with 35-40% erucic acid.

Plants were grown in a glasshouse and hybrids obtained through controlled pollinization. Staining of somatic chromosomes was carried out using the Satyanarayana and Sen Method (1973). Meiotic analyses were performed in MI cells stained with alcoholic-chloridric-carmine (Snow, 1963). In order to estimate hybrids and derivatives pollen fertility, pollen grains were stained with cotton blue-lactophenol dye. Female fertility was estimated counting the number of seeds in crosses where the hybrids and derivatives were used as females. Seed oil fatty acid composition was determined by means of gas chromotography of methil esters prepared by standard procedures. As genetical markers we used the genes responsible for low erucic acid content in the seed oil. These genes are located in each Brassica genome (Fernández-Escobar et al., 1988). The genes controlling the synthesis of erucic acid are represented by E. The mutant alelles which control the low erucic acid content (<1%) are represented by E_o and those for the high erucic content by E_1.

Meiotic analyses were carried out in the three possible interspecific hybrids combining two cultivated tetraploid Brassica species which always have two homologous and two homoeologous genomes. In meiotic analyses performed by other authors (Sasaoka, 1930; U, 1935; Rouselle and Eber, 1983,...) no distinction was made between bivalent rings and rods. It is however essential to distinguish between the two in order to obtain a correct meiotic interpretation. The MI were analysed for every interspecific hybrid and the maximum pairing rate per chromosomic arm "c" (Kimber et al., 1981; Alonso and Kimber, 1981; Kimber and Alonso, 1981) was calculated. In order to calculate this variable we assumed that

all chromosomes of interspecific hybrids participate to some extent in the pairing. The value of "c" may vary between zero and one; while zero represents the absence of pairing, one represents the existence of pairing in all chromosome arms. The value "c" is an estimation of the mean arm pairing frequency. It is not possible to calculate the preferential pairing tendency "x" in hybrids with genomes possessing a different number of chromosomes. In order to estimate the homoeologous pairing rate, a calculation was carried out based on 2:1:1 pairing type corresponding to the genomic constitutions AABC, ABCC and ABBC of interspecific Brassica hybrids. For this calculation we assumed that only homologous genomes pair. It is necessary to calculate a new value for "c", termed "c2" from now on. If "c2" is greater than one, this implies that homoeologous pairing has taken place.

RESULTS AND DISCUSSION

1) Meiotic pairing of interspecific hybrids.

The results of meiotic pairing of hybrids between cultivated tetraploid Brassica species, the values of "c" and "c2" and the expected pairing according to the model 2:1:1 appear in Table 1.

The most frequent meiotic configuration in hybrids between B. napus and B. juncea (AABC) is 10 II + 17 I (76% of the MI), with most of the bivalents being rings. The estimated pairing according to the model 2:1:1 is very close to that observed, and c2 is not greater than one, thus indicating that in these hybrids the A genomes dominate to a large extent the chromosomic pairing, making the pairing A-C, A-B and B-C very infrequent. About 10% of the cells in AI have 10 chromosomes in each pole and 17 chromosomes in the equator. One to six microspores of different size were produced by each mother cell.

Hybrids between B. napus and B. carinata (ABCC) show a c2 value of over one. The average of bivalents per cell is 10.5. These results along with the presence of trivalents indicate that the homoeologous pairing (A-B, A-C and B-C) is greater than in the AABC hybrids, although not notably high. The ABCC hybrids also present anaphases with central chromosomes which do not emigrate to the poles. Two to six microspores of different size were produced by each mother cell.

Hybrids between B. juncea and B. carinata show a very high degree of pairing, with a c2 value of 1.66 and an average of 14.8 bivalents per cell. This suggests that the homoeologous pairing is very high. Given the absence of trivalents, this pairing is probably type A-C. The anaphases I are more regular than previous hybrids. The sporads stage was regular and the size of pollen grains practically homogeneous.

2) Genetic transfer of B. napus and B. carinata.

Hybrids between B. napus and B. carinata were backcrossed to B. carinata. Two types of backcrosses were carried out using the F1 either as female or as male. In order to avoid nucleo-citoplasmic interactions the BC1 with the citoplasm of B. napus were not obtained. Twelve BC1 plants of BR x F1 type and three of the F1 x BR type were studied. The first ones had between 33 and 35 chromosomes, a high meiotic pairing rate (16.21 bivalents/cell), 78% pollen fertility and 32% female fertility. BC1 plants of the F1 x BR type had 36, 37 and 38 chromosomes respectively, a pollen fertility of 24% and a female fertility of 4.2%. These results indicate that in order to recover the genomic constitution and fertility of B. carinata, the F1 between B. napus and B. carinata must be the male in the backcrosses. Of the 15 BC2 plants studied, 14 had 34 chromosomes and one 35. The average pollen viability was 96% and female fertility 83%.

Following this route, seeds containing a minimum erucic acid content of 23% were obtained, probably with an E_1E_1EoEo or $EoEoE_1E_1$ constitution.

These intermediate erucic acid content derivatives have probably arise from genetic transfer of Eo alleles from the C genome of B. napus to B. carinata.

3) Genetic transfer from B. juncea to B. carinata.

Hybrids between B. juncea and B. carinata were backcrossed to B. carinata. 17 BC1 plants were analysed, all with the citoplasm of B. carinata. 16 of these had 34 chromosomes and one 17. Apart from this last one, all plants were vigorous, with morphology similar to B. carinata. The meiosis shows a great regularity with an average of 16.7 bivalents per cell, although with occasional univalents and quadrivalents. No significant differences exist between plants resulting from BR x F1 or F1 x BR crosses. Its average pollen viability is 91% and female fertility 77%. It is possible to obtain BC1 plants similar to B. carinata of great fertility and meiotic regularity. BC1 plants produced BC2 plants quite close to B. carinata in all the characters. Selfing of BC2 plants has provided seeds with a minimum of 13.5% erucic acid in the seed oil. This seed probably possesses an $E_1EoEoEo$ or $EoEoE_1Eo$ constitution.

The high pairing rate of hybrids between B. juncea and B. carinata allows the recombination of chromosomes of different genomes, A, B and C. Thus, by means of a single route, desirable traits could be transfer from the three basic Brassica genomes to B. carinata.

4) Joint genetic transfer of B. napus and B. juncea to B. carinata.

In order to obtain forms of B. carinata with B. napus and B. juncea genes, plants of the aforementioned routes may be crossed. For this reason BC1 plants obtained from the first route and BC2 plants from the second were crossed, although it would have been better to cross BC1 plants from both routes. Plants produced by the selfing of these F1 showed a high meiotic regularity. It was possible to isolate among them "zero erucic" plants. Genetic transfer may have occurred either from the B. juncea B genome and B. napus C genome to B and C of B. carinata or else from the B. juncea A and B genomes to C and B of B. carinata respectively.

5) Simultaneous genetic transfer from B. napus and B. juncea to B. carinata.

The transfer of B. napus and B. juncea genes to B. carinata may be attempted in a single breeding programme which simplifies the process considerably. The F1 between B. napus and B. juncea was crossed with B. carinata. Two types of crosses were carried out, using F1 as female or as male. Six BR x (BN x BJ) type plants were studied. Four of these had 34 chromosomes, one 33 and the other 35. These plants showed an average of 16.31 bivalents in MI (Table 2). Their pollen fertility was 80% and female fertility was 36%. Five of the (BJ x BN) x BR type plants were analysed. Three had 34 chromosomes, one 33 and another 37. These plants showed an average of 11.35 bivalents and 9.18 univalents in MI (Table 2). The pollen fertility was 4% and their female fertility was 4.8%. Only BC1 and BC2 descendents of the BR x F1 type plants were analysed. These showed a great meitoic regularity (Table 2), a high pollen viability, 94.3 and 97.4 respectively, a good female fertility and a morphology similar to B. carinata. Seeds with a minimum of 9% erucic acid content in the selfing seed of BC1 plants were isolated, probably with the constitution $EoEoE_1Eo$ or $E_1EoEoEo$. The isolation of seeds with erucic acid content reduced to a fourth of the normal values for B. carinata confirms the posibility of transfering genes from the hybrid between B. napus and B. juncea to B. carinata.

The most striking feature of this route is the difference existing between the three-way hybrids of the type BR x F1 and F1 x BR. Hybrids

between B. napus and B. juncea occasionally appear to produce gametes with 17 + 1 chromosomes and BC genomic constitution, resulting from the formation of a nucleus of partial restitution in AABC hybrids. These gametes seem to compete better than the rest in the fertilization of B. carinata eggcells when AABC hybrids are used as males. Failure in pairing is the best stimulus for the formation of restitution nuclei (Hermsen, 1984). Gametes with only some of the interspecific hybrid genomes have been described in Brassica (Nawankity, 1970) and in other species (Alonso and Kimber, 1984).

CONCLUSIONS

- Hybrids between B. napus and B. juncea, with a genomic constitution of AABC, present an extremely low homoeologous pairing A-B, A-C and B-C, restricted to a sporadic open bivalent or rod, while homologous pairing A-A occurs in most cases. Genetic transfer between A, B and C genomes is therefore practically non-existent.
- Hybrids between B. napus and B. carinata, with a genomic constitution of ABCC, show some homoeologous pairing, A-B, A-C and B-C. Transfer between A, B and C genomes is therefore possible although quite infrequent.
- Hybrids between B. juncea and B. carinata, with a genomic constitution of ABBC, show a high degree of both homologous and homoeologous pairing, the latter probably of the A-C type which allows the genetic transfer between both A and C genomes.
- In order to transfer genes from B. napus to B. carinata, hybrids of both species must be used as male in backcrosses with B. carinata. The second generation of backcrossing produces plants which are essentially B. carinata types. If a rigorous selection is carried out, fertile plants may be isolated with a high pairing rate between plants produced in the first generation of backcrossing.
- In order to transfer genes from B. juncea to B. carinata F1 hybrids between both species may be used either as male or as female in bakcrosses with B. carinata. It is relatively easy to isolate fertile BC1 plants among the above backcrosses. The BC2 generation provides B. carinata derivatives with some genes from B. juncea. B. carinata derivatives are easilly recoverd from B. juncea than B. napus.
- The recovery of B. carinata derivatives with both B. napus and B. juncea genes may be obtained through the crossing of first generation backcrossed plants which used the genetical transfer routes of B. napus and B. juncea to B. carinata, followed by one or two selfing cycles.
- Genetic transfer from B. napus and B. juncea to B. carinata may be also carried out simultaneously. Hybrids between B. napus and B. juncea, with genomic constitution of AABC, produce partially reduced gametes with a BC genomic constitution. There is strong competitiveness among the pollen grains over the fertilization of B. carinata eggcells, and those pollen grains with a genomic constitution similar to BC and with low levels of aneuploidies fertilize them selectively. The result of this fertilization is the production of semisynthetic forms of B. carinata which, following a single bakcross, recover all the morphological characteristics and fertility of B. carinata.

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Table 1: Meiotic pairing of interspecific hybrids among cultivated tetraploid Brassica species

GENOMIC CONSTITUTION (CHROM. Nº)	CROSS TYPE	Nº CELLS ANALYSED	I $\bar{X}$	I INTERVAL	III $\bar{X}$	III INTERVAL	II $\bar{X}$	II INTERVAL	TOTAL II $\bar{X}$	TOTAL II INTERVAL	III $\bar{X}$	III INTERVAL	c	c2
AABC	BJ x BN	35	16,48	13-19	2,37	1-5	8,06	5-10	9,84	9-10				
(37)	BN x BJ	13	17,3	17-19	1,31	1-3	8,54	7-10	10,26	9-12				
	MEAN	48	16,89	13-19	1,74	1-5	8,30	5-10	10,05	9-12			0,51	0,92
	EXP MOD (1)		17,12		1,50		8,40							
ABCC	BN x BR	75	15,53	10-20	0,84	0-3	9,34	6-13	10,09	6-13	0,88	0-3		
(36)	BR x BN	20	15,85	12-18	1,50	0-3	8,25	8-12	10,75	8-12	0,05	0-1		
	MEAN	95	15,69	10-20	1,17	0-3	9,30	6-13	10,42	6-13	0,06	0-3	0,58	1,11
	EXP MOD (1)		18,0		0		9,00				0			
ABBC	BJ x BR	46	15,39	1-11	2,96	1-6	11,85	9-14	14,80	12-17			0,78	1,56
(35)	EXP MOD (1)				0		8,00							

BN= B. napus BR: B. carinata BJ: B. juncea

(1) Exp.Mod.= Expected pairing if only homologous chromosomes are able to pair

Table 2: Meiotic pairing of the plants of the simultaneously genetic transfer route from B. napus to B. carinata

CROSS TYPE	Nº cells analysed	I $\bar{X}$	I INTERVAL	III $\bar{X}$	III INTERVAL	II $\bar{X}$	II INTERVAL	III $\bar{X}$	III INTERVAL	IV $\bar{X}$	IV INTERVAL
THREE WAY HYBRIDS											
BR x F1 BR x (BJ x BN) or BR x (BN x BJ)	121	1,0	0-6	1,06	0-4	15,25	10-17	0,1	0-1	0,03	0-1
F1 x BR (BJ x BN) x BR	38	9,18	2-18	1,95	0-6	10,41	8-14	0,03	0-1	0	--
BC1	267	0,05	0-2	0,41	0-4	16,53	13-17	0,02	0-2	0	--
BC2	58	0	--	0,64	0-4	16,33	13-17	0	--	0,01	0-1
BR	58	0	--	0,86	0-4	16,13	15-17	0	--	0	--

BR: B. carinata BR: BN: B. napus BJ: B. juncea