INTERSPECIFIC CROSSING OF BRASSICA CARINATA AND B. OLERACEA FOR BREEDING YELLOW-SEEDED B. OLERACEA/B. NAPUS

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INTRODUCTION

Yellow seed in Brassica oil crop species due to its thinner seed coat has the distinct advantage of an increased oil and protein content along with lower fibre in the meal over its black-seeded counterpart (Stringam et al. 1974; Woods 1980; Shirzadegan and Röbbelen 1985; Daun and DeClercq 1988). Yellow seed coat is partially digestible, whereas brown/black seed coat is completely indigestible (Bell and Shires 1982). Different approaches of interspecific crossing for breeding yellow-seeded B. napus have been considered (Hou-Li and Yong-Tong 1987; Barcikowska et al. 1987; Zaman 1988; Chen et al. 1989). The main difficulty of breeding yellow-seeded B. napus is the non-availability of yellow-seeded CC genomic species. Consequently, breeding yellow-seeded CC genomic species was considered an intermediate step towards breeding yellow-seeded B. napus. In our study, we aimed to create yellow-seeded CC genomic species by crossing yellow-seeded B. carinata (BBCC) and black-seeded B. oleracea (CC). If achieved, it would be a novel horizon of breeding yellow-seeded B. napus.

MATERIALS AND METHODS

The research work was perfomed at MARIBO seed. Two black-seeded CC genomic genotypes, viz. B. alboglabra (a form of B. oleracea, Prakash and Hinata 1980) and rapid-cycling B. oleracea, and one yellow-seeded BBCC genomic genotype B. carinata were used. All plant material was grown in growth chamber: day temperature 20°C, night temperature 15°C, diurnal period 16 hours. Reciprocal crosses between B. carinata and B. alboglabra, and single directional cross between rapid-cycling B. oleracea and B. carinata were made. Embryo rescue technique developed for Brassica at MARIBO seed was used for the production of F_1 hybrids when B. alboglabra/B. oleracea were used as female parent. For this purpose, immature siliquae were harvested 20 to 36 days after pollination. Fertilized ovules were excised and hybrid embryos were rescued. They were cultured in vitro, and plants were regenerated.

F_1 plants of all the crosses were grown in glasshouse and were selfed by hand (bud-pollination) for the production of F_2 seeds. F_1 plants were also backcrossed with B. alboglabra. F_2 and backcross populations were grown in glasshouse and were selfed by bag isolation. Flower colour, pollen fertility and different morphological characters of F_1, F_2 and backcross populations were recorded. Per cent siliqua set was estimated based on the number of siliqua set in relation to the number of bag-isolated buds. Pollen fertility was estimated by acetocarmine squash technique.

RESULTS

A strong reciprocal crossability barrier was found between BBCC genomic species B. carinata and CC genomic species B. alboglabra. Using B. carinata as female and B. alboglabra as male in the cross 7.8 seeds per pollination were harvested (Table 1). The F_1 seeds were brown in colour

Table 1. Crossability between B. carinata and B. alboglabra/rapid-cycling B. oleracea in three combinations.

Cross	No. pollinations	Per cent siliqua set	No. seeds per pollination	No. aborted seeds per pollination
B. alboglabra x B. carinata	87	100	-	5.8
B. carinata x B. alboglabra	46	100	7.8	2.7
B. oleracea x B. carinata	60	100	-	6.2

and smaller in size: 1000-seed weight= 1.005 g as compared to the selfed seeds of the mother plant B. carinata which were yellow and had a 1000-seed weight= 4.002 g. The reciprocal cross, i.e. CC x BBCC, failed to produce any seed. Counts on the number of aborted seeds per pollination suggested that 5.8 ovule per ovary of B. alboglabra and 6.2 ovules per ovary of rapid-cycling B. oleracea were fertilized after pollination with B. carinata. But all the hybrid embryos degenerated before maturity. By embryo rescue technique 24 hybrids of B. alboglabra x B. carinata and 3 hybrids of rapid-cycling B. oleracea x B. carinata were produced.

Hybrids of all the crosses were easily distinguished from their respective parents by their leaf shape and flower colour. Both B. alboglabra and rapid-cycling B. oleracea were white-flowered and B. carinata was yellow-flowered. Flower colour of the F_1 plants were creamy-white. The leaf shape of the hybrids were intermediate of the two parents (Table 2).

Table 2. Characteristics of F_1 hybrids of black-seeded B. alboglabra/rapid-cycling B. oleracea and yellow-seeded B. carinata

F1 hybrids	No. plants studied	Leaf shape	Flower colour	Per cent fertile pollen
B. alboglabra x B. carinata	24	interme-diate	creamy white	6.4
B. carinata x B. alboglabra	47	"	"	5.1
B. oleracea x B. carinata	3	"	"	1.4
Total	74	interme-diate	creamy white	4.3

Reciprocal difference was observed in the F_1 hybrids in respect of fertility. The hybrids of B. alboglabra x B. carinata produced 9 viable plus 75 aborted F_2 seeds per 1000 bud-pollinations (Table 3a). After backcrossing with B. alboglabra, 348 viable plus 741 aborted backcross seeds per 1000 pollinations were harvested (Table 3b). On the other hand, the hybrids of the reciprocal cross, i.e. B. carinata x B. alboglabra, produced 8 viable plus 22 aborted F_2 seeds per 1000 bud-pollinations; and 209 viable plus 252 aborted backcross seeds per 1000 pollinations with B. alboglabra. In all cases, backcrossing of hybrids with B. alboglabra gave higher seed setting compared to selfing. A significant number of F_2

Table 3. Efficiency of selfing and the efficiency of backcrossing of the F_1 hybrids of black-seeded B. alboglabra/rapid-cycling B. oleracea and yellow-seeded B. carinata.

a. Efficiency of selfing

F1 hybrids	No. bud pollinations done	Per cent siliqua set	No. seeds per pollination	No. aborted seeds per pollination
B. alboglabra x B. carinata	1399	6.4	0.009	0.075
B. carinata x B. alboglabra	2205	2.8	0.008	0.022
B. oleracea x B. carinata	90	0.0	0.0	0.0
Total	3694	4.1	0.008	0.042

b. Efficiency of backcrossing with B. alboglabra

F1 hybrids	No. pollinations done	Per cent siliqua set	No. seeds per pollination	No. aborted seeds per pollination
B. alboglabra x B. carinata	610	41.3	0.348	0.741
B. carinata x B. alboglabra	846	29.8	0.209	0.252
B. oleracea x B. carinata	72	13.9	0.097	0.139
Total	1528	33.6	0.259	0.442

and backcross embryos degenerated before maturity as indicated by the number of aborted seeds per pollination. In general, the fertility of all the hybrids was low. Among the three crosses, the hybrids of rapid-cycling B. oleracea x B. carinata had the lowest fertility.

From the above crosses, a total of 29 F_2 plants were grown (Table 4). All the F_2 plants were morphologically more close to B. carinata than to B. alboglabra. Pollen fertility in the F_2 population varied among plants from zero to 65.3%. Only 11 plants produced seeds under bag isolation. Among these plants, per cent siliqua set varied from 7.8 to 63.6 with 0.2 to 6.0 seeds set per siliqua. Of these 11 plants, 2 produced yellowish-brown, 1 light brown and the remaining 8 plants produced black seeds.

Abundant B. alboglabra/B. oleracea type plants were obtained in backcross populations. Pollen fertility among plants in backcross populations varied from zero to 82.2% (Table 4). Out of the 172 backcross plants of the three crosses 31 plants produced seeds under bag isolation. Of the 28 backcross plants derived from B. alboglabra x B. carinata and the reciprocal cross, the per cent siliqua set varied from 2.5% to 68.6% and seed set per siliqua from 0.2 to 30.7. Three plants produced light brown seeds and the remaining 25 plants produced black seeds. It was found that these 3 plants produced 3.6, 12.0 and 13.8 per cent siliqua with 0.7, 1.3 and 2.0 seeds per siliqua. The 3 backcross plants derived from the cross rapid-cycling B. oleracea x B. carinata showed good siliqua and seed set (Table 4). However, all of these 3 plants produced black seeds.

Table 4. Pollen fertility, siliqua set, seed set and seed coat colour of F_2 and backcross populations of the crosses between black-seeded B. alboglabra / rapid-cycling B. oleracea and yellow-seeded B. carinata

Parentage and populations	No. plants grown	Pollen fertility		No. plants produced seed	Per cent siliqua set		No.seeds/siliqua		Seed coat colour		
		Range	Mean		Range	Mean	Range	Mean	Yellowish brown	Light brown	Black
F2 populations:											
albo x cari*	11	0.0-58.2	17.4	4	7.8-52.1	25.9	0.2-5.6	1.8	-	1	3
cari x albo	18	0.0-65.3	24.0	7	18.2-63.6	44.4	0.3-6.0	2.6	2	-	5
Total	29	0.0-65.3	21.5	11	7.8-63.6	40.0	0.2-6.0	2.5	2	1	8
Backcross populations											
(albo x cari) x albo	86	0.0-82.2	19.1	16	2.5-68.6	30.3	0.2-30.7	6.9	-	2	14
(cari x albo) x albo	79	0.0-77.0	10.0	12	13.8-65.2	34.2	0.5-12.0	4.7	-	1	11
(ole** x cari) x albo	7	0.0-80.1	18.7	3	57.1-76.2	73.0	20.3-29.3	24.4	-	-	3
Total	172	0.0-82.2	14.9	31	2.5-76.2	35.2	0.2-30.7	7.7	-	3	28

* albo = B. alboglabra, cari = B. carinata

** ole = B. oleracea

DISCUSSION

The amphidiploid species B. carinata (BBCC) possesses the genome of B. nigra (BB) and B. oleracea (CC). Therefore, it has been assumed that the yellow-seeded B. carinata in its CC genome carries the gene(s) for yellow seed coat character. Interspecific crossing between yellow-seeded B. carinata and black-seeded B. oleracea was done by other's (Barcikowska et al. 1987; Zaman 1988) aiming at breeding yellow-seeded B. oleracea. However, limited success was achieved so far. Zaman (1988) reported that the F_2 population derived from the cross B. carinata x B. alboglabra were all B. carinata type with chromosome number 2n=34. However, he did report light-brown-seeded B. alboglabra following selfing of BC_1 (F_1 x B. alboglabra) plants.

In the F_2 populations of the present study, yellowish-brown- and light-brown-seeded plants, and in backcross populations light-brown-seeded plants were obtained. These plants constitute the first step towards breeding yellow-seeded B. oleracea. Continued selection in the following generations is necessary.

Strong reciprocal crossability barrier exists between CC and BBCC genomic species. Different reasons of interspecific crossability barrier have been accounted in the literature. After fertilization of the egg cell by the male gamete, degeneration of the hybrid embryo due to the lack of endosperm development occurs in many interspecific crosses (Singh et al. 1990). Different in vitro methods, e.g. embryo rescue, ovule culture, ovary culture, protoplast fusion etc., have proved useful in wide hybridization of plants. Also in the present case, application of embryo rescue technique was necessary in order to obtain hybrids of CC x BBCC cross.

The F_1 hybrid plants of CC x BBCC and of the reciprocal cross did not appear different in respect of morphological characters. However, difference was found with respect to fertility. In the F_1 plants of CC x BBCC, a higher proportion of ovules were fertilized after self-pollination and after cross-pollination with B. alboglabra than in the reciprocal cross. Therefore, as the chromosome constitution of the reciprocal crosses were equal, a cytoplasmic difference was indicated. Based on fraction I protein data, Uchimiya and Wildman (1978) drew the conclusion that B. nigra functioned as female parent during the spontaneous evolution of B. carinata. Accepting this conclusion, the cytoplasm in the present F_1 plants of CC x BBCC was B. oleracea type; while cytoplasm in the F_1 plants of BBCC x CC was B. nigra type. Further studies have shown these two types of cytoplasm also to be distinctly different from each other in respect of chloroplast and mitochondrial DNA (Palmer et al. 1983; Palmer 1988). Thus, it is concluded that the differences in cytoplasm caused the differences in fertility observed in the present study.

SUMMARY

Reciprocal interspecific crosses between yellow-seeded B. carinata (BBCC) and black-seeded B. oleracea (CC) were done to create B. oleracea with yellow seed coat. Significant reciprocal crossability barriers were observed between the BBCC and CC genomic species. Using B. carinata as female, 7.8 hybrid seeds per pollination were obtained. The reciprocal cross failed to produce seed, but 27 hybrid plants were obtained by embryo rescue technique. Reciprocal differences were found also in F_1 plants with respect to fertility. Fertility of all F_1 hybrids was very low: 8 seeds per 1000 self-pollinations (bud-pollination). After backcrossing the F_1 hybrids with B. oleracea 259 seeds per 1000 pollinations were obtained. In the F_2 populations 38% and in the backcross populations 18% of the plants produced seeds under bag isolation. Out of 11 F_2 plants 2 had yellowish-brown seed, and out of 31 backcross plants 3 had light-brown seed.

REFERENCES

BARCIKOWSKA, B., ZWIERZYKOWSKA, E. and BALICKA, M. 1987. On the way to yellow seeded Brassica napus L. - Hybrids of B. campestris x B. oleracea and of B. oleracea x B. carinata yellow seeded. Proc. 7th Int. Rapeseed Cong., Poznan, Poland. Volume 2, pp. 492-495.

BELL, J.M. and SHIRES, A. 1982. Composition and digestibility by pigs of hull fractions from rapeseed cultivars with yellow or brown seed coats. Can. J. Anim. Sci. 62: 557-565.

CHEN, B.Y., HENEEN, W.K. and JÖNSSON, R. 1988. Resynthesis of Brassica napus L. through interspecific hybridization between B. alboglabra Bailey and B. campestris L. with special emphasis on seed colour. Plant Breed. 101: 52-59.

DAUN, J.K. and DeCLERCQ, D.R. 1988. Quality of yellow and dark seeds in Brassica campestris Canola varieties Candle and Tobin. J. Am. Oil Chem. Soc. 65: 122-126.

HOU-LI, L. and YONG-TONG, G. 1987. Some fundamental problems conducted from the studies on the breeding yellow-seeded Brassica napus L. Proc 7th Int. Rapeseed Cong., Poznan, Poland. Volume 2, pp. 476-480.

PALMER, J.D. 1988. Intraspecific variation and multicircularity in Brassica mitochondrial DNAs. Genetics 118: 341-351.

PALMER, J.D., SHIELDS, C.R., COHEN, D.B. and ORTON, T.J. 1983. Chloroplast DNA evolution and the origin of amphidiploid Brassica species. Theor. Appl. Genet. 65: 181-189.

PRAKASH, S. and HINATA, K. 1980. Taxonomy, cytogenetics and origin of crop Brassicas, a review. Opera Bot. 55: 1-57.

SHIRZADEGAN, M. and RÖBBELEN, G. 1985. Influence of seed color and hull proportion on quality properties of seeds in Brassica napus L. Fette. Seifen. Anstrichm. 87: 235-237.

SINGH, A.K., MOSS, J.P. and SMARTT, J. 1990. Ploidy manipulations for interspecific gene transfer. Advances in Agron. 43: 199-240.

STRINGAM, G.R., McGREGOR, D.I. and PAWLOWSKI, S.H. 1974. Chemical and morphological characteristics associated with seedcoat color in rapeseed. Proc. 4th Int. Rapeseed Conf., Giessen, W. Germany. pp. 99-108.

UCHIMIYA, H. and WILDMAN, S.G. 1978. Evolution of fraction I protein in relation to origin of amphidiploid Brassica species and other members of the Cruciferae. J. Hered. 69: 299-303.

WOODS, D.L. 1980. The association of yellow seed coat with other characters in mustard B. juncea. Cruciferae Newsl. 5: 23-24.

ZAMAN, M.W. 1988. Limitations for introgression of yellow seed coat colour in Brassica napus. Sveriges Utsädesf. Tidskr. 98: 157-161.