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Synthesis of *Brassica carinata* from *Brassica nigra* × *Brassica oleracea* hybrids obtained by ovary culture

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Summary. *Brassica carinata* was synthesized by hybridization between its diploid progenitor species *B. nigra* and *B. oleracea* followed by chromosome doubling. Up to 40 times more hybrids were obtained, using ovary culture than with conventional hand pollination. Two hybrids from the cross *B. nigra* × *B. oleracea* var 'alboglabra' were raised to maturity. High chromosome pairing was observed in both the hybrids and the amphidiploid. A range of variability for desirable characters is reported in the synthesized amphidiploids.

Key words: *Brassica carinata* – Synthesis – Ovary culture – Cytology – Variability

Introduction

Brassica carinata (2n = 34, genome BBCC) is a natural amphidiploid derived from hybridization between *B. oleracea* (2n = 18, CC) and *B. nigra* (2n = 16, BB), It is cultivated in Ethiopia and Kenya as an oilseed, vegetable and fodder crop (Tcacenco et al. 1985). Because of its high tolerance to aphids and resistance to *Alternaria* blight, white rust and other common fungal diseases affecting rapeseed mustard (Kolte 1985; Anonymous 1987), attempts are being made to introduce *B. carinata* into India (Raut and Prakash 1985; Katiyar et al. 1986). However, the adaptation of *B. carinata* to alien environments and its improvement have been restricted by the limited variability of available cultivars (Narsimhulu and Chopra 1987).

Artificial synthesis of the amphidiploid by hybridization between its progenitors followed by chromosome doubling provides a means to increase the usable genetic variability. This has been well demonstrated by the successful synthesis and subsequent adaptation of the related amphidiploid *B. napus* (2n = 38, AACC; Olsson and Ellerstrom 1980; Namai et al. 1980). However, attempts to synthesize *B. carinata* have been few, and even these were mainly concerned with establishing cytogenetical relationships between the diploids and the amphidiploids (Frandsen 1947; Mizushima and Katsuo 1953).

Conventional hand pollination rarely leads to interspecific hybrids. Even when such hybrids can be obtained, their frequency is low. In vitro techniques such as ovary, ovule and embryo culture have been employed to obtain a high frequency of hybrids between *B. campestris* (2n = 20, AA) and *B. oleracea* in the synthesis of *B. napus* (Inomata 1978; Takeshita et al. 1980; Gland 1982). These techniques have not been previously used for the synthesis of *B. carinata*. In *B. nigra* × *B. oleracea* crosses, rescue of hybrids by ovule or embryo culture is difficult because of the very small size of the *B. nigra* ovules and the specific osmotic and nutritional requirements of the young embryos (Raghavan 1986). Ovary culture is relatively less complicated. This paper presents the results of a study on the use of ovary culture in the synthesis of *B. carinata* from the two interspecific crosses: (1) *B. nigra* × *B. oleracea* var 'alboglabra', and (2) *B. nigra* × *B. oleracea* var 'capitata'. Chromosomal associations in the hybrids and the variability generated in the synthesized *B. carinata* were also examined.

Materials and methods

Two varieties of *B. oleracea*, var 'alboglabra' (kale) and var 'capitata' (cabbage), were crossed with one variety of *B. nigra*, which was the female parent in each case. Pollinations were carried out on field-grown plants. Flower buds, emasculated 1 day before anthesis, were pollinated the next day with pollen from newly dehisced anthers.

For ovary culture, a few pollinated ovaries were excised along with the pedicel 6 and 10 days after pollination, respec-

Table 1. Comparison of the frequencies of hybrids obtained by hand pollination and by ovary culture

Cross	Hand pollination		Ovary culture		
	No. of pollinations	No. of hybrids (%)	No. of ovaries cultured	Days after pollination	No. of hybrids (%)
B. nigra × *B. oleracea* var 'alboglabra'	225	1 (0.4)	24	6	4 (16)
B. nigra × *B. oleracea* var 'capitata'	138	1 (0.7)	30	10	3 (10)

Table 2. Morphological characters of parents, hybrids and synthesized *B. carinata*

Characters	Parents		Hybrids		Synthesized[a] *B. carinata*
	B. nigra	*B. oleracea* var 'alboglabra'	1	2	
Plant height (cm)	155	111	153	115	82 – 241
No. of primary branches	11	7	9	14	7 – 24
No. of secondary branches	21	0	32	30	0 – 92
Length of primary fruiting axis (cm)	26	51	42	95	6 – 75
Pod density (per cm primary axis)	1.6	0.8	0.5	0.9	0.2 – 1.3
Pod length (cm)	1.4	9.0	2.5	2.0	1.3 – 5.8
No. of seeds/pod	6	26	2	–	1 – 23
Pollen fertility (%)	92	90.5	36.5	–	48.3 – 94.2
Days to flowering	70	90	95	110	68 – 110
Length of petals (cm)	0.9	1.8	1.2	1.1	0.4 – 1.5
Width of petals (cm)	0.3	1.1	0.6	0.6	0.4 – 0.7
Flower colour	Yellow	White	Yellowish white	Yellow	Yellowish white to yellow

[a] Range of variation in 40 amphidiploid plants that were progeny of amphidiploid 1

tively, for the two crosses. They were 1.5–2 cm long at the time of culture. The ovaries were surface sterilized in 2% mercuric chloride, washed with sterile water and implanted vertically in agarified Murashige and Skoog's (1962) medium supplemented with 0.5% sucrose, 2 ppm kinetin (Kn) and 1 ppm naphthaleneacetic acid (NAA). A 16 h light regime was alternated with an 8 h dark period during ovary culture. The ovaries were dissected out after 30 days, or earlier if they had begun to turn brown. Seeds thus obtained were germinated in MS medium without supplements. F_1 seedlings were transferred to pots and then to soil; some seedlings did not survive the transfer.

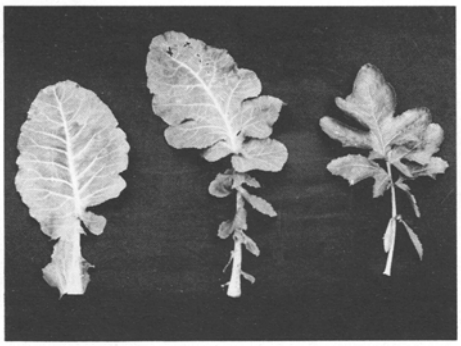
Colchicine (0.5%) was applied on 5 consecutive days to shoot spices of the hybrids at the vegetative stage to induce chromosome doubling. The pollen fertility of the hybrids was determined by staining pollen from mature, undehisced anthers with 1% acetocarmine containing 10% glycerol. For the cytological analysis, pollen mother cells, (PMCs) were studied using the routine aceto-carmine squash technique. Seeds obtained from the doubled hybrid (amphidiploid 1) were sown in order to study the variability of the amphidiploids obtained by artificial synthesis.

Results

For the two crosses, hand pollination alone yielded hybrids at a frequency of 0.4% and 0.7%, while ovary culture increased the frequency to 16% and 10% respectively (Table 1). In all, 7 hybrid seedlings were obtained by ovary culture of which two, hybrid 1 and hybrid 2, were grown to maturity. Both were from the cross *B. nigra* × *B. oleracea* var 'alboglabra'.

Both hybrids were intermediate between the parents for general morphological characters (Table 2, Figs. 1, 2). Hybrid 1 showed anthocyanin pigmentation in the leaf axis at the seedling stage. The leaves were deeply lobed as in *B. nigra* and glaucous as in *B. oleracea*. While hybrid 1 had yellowish white flowers, hybrid 2 had deep yellow flowers.

Colchicine application led to chromosome doubling in hybrid 1 (which is, therefore, referred to as amphidiploid 1), but not in hybrid 2. The cytological examination of 50 PMCs of hybrid 2 showed 17 chromosomes present as 3II + 11I in 60% of the cells (Fig. 3). The maximum association observed was 1III + 5II + 4I in which at least two bivalents did not appear to be true chiasmate associations but side-to-side secondary associations. Amphidiploid 1 had 34 chromosomes. At metaphase I, the chromosomes were most frequently present as 17 bivalents (66% of the cells). The configuration 3IV + 11II was observed in 28% of the cells, some of which were at diakinesis, and configuration 2IV + 13II in 6% of the cells.



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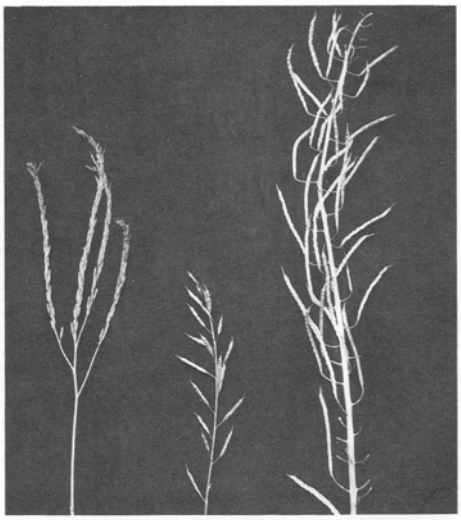

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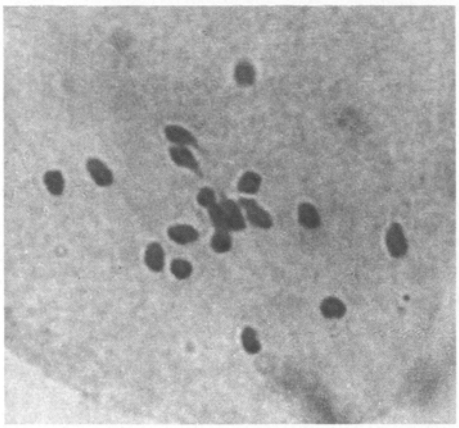

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Figs. 1–3. **1** Leaf of hybrid 1 (middle) and its parents *B. oleracea* var 'alboglabra' (left) and *B. nigra* (right). **2** Pod-bearing branch of hybrid 1 (middle), *B. nigra* (left) and *B. oleracea* var 'alboglabra' (right). **3** Late metaphase in hybrid 2 showing 3II+11I (two bivalents precociously terminalized) (×4,000)

Pollen fertility was 36.5% in amphidiploid 1; hybrid 2 had very little pollen and that was sterile. This was reflected in the seed set. While seeds set on amphidiploid 1, no seeds were obtained from hybrid 2. Upon germination, seeds from amphidiploid 1 gave rise to vigorous seedlings: 40 mature amphidiploid plants (*B. carinata*) were subsequently analysed for morphological characters. The range of variability observed among these plants for morphological and yield-contributing characters is presented in Table 2.

Discussion

In crosses where the embryo degenerates at an early stage and the ovules are too small to be excised intact, the in vitro culture of pollinated ovaries is a relatively simple technique with which to rescue hybrids. Our results indicate that in *B. nigra* × *B. oleracea* crosses, where seed set is otherwise very low, it is possible to obtain a reasonably high frequency of hybrids by resorting to ovary culture. The increase in the frequency of hybrids, when compared with hand pollination, was 40 times higher in the cross *B. nigra* × *B. oleracea* var 'alboglabra' and 14 times higher in the cross *B. nigra* × *B. oleracea* var 'capitata'. These hybrids have not been reported before. Inomata (1978) reported similar results for the synthesis of the related amphidiploid, *B. napus*. He obtained hybrids from the cross *B. campestris* × *B. oleracea* at frequencies ranging from 3% to 23% by ovary culture when hand pollination yielded less than 1% hybrids.

Kinetin and NAA are known to have a salutary effect on hybrid embryo development (Nesling and Morris 1979; Sarla 1988). In this study, 2 ppm kinetin and 1 ppm NAA were used. A range of hormones and their concentrations, and other supplements need to be evaluated for each cross to determine the optimum conditions for obtaining a higher frequency of interspecific hybrids.

Pollen and seed sterility is to be expected in interspecific hybrids. However, the doubling of the chromosome number generally restores fertility if there is strict homologous pairing. In the present study, a maximum of 3IV in the amphidiploid indicated that diploid chromosome behaviour was not fully achieved by merely doubling the chromosome number. Mizushima and Katsuo (1953) reported 4IV in synthesized *B. carinata*. The general occurrence of multivalents in resynthesized amphidiploids has also been reported in *B. napus* (Attia and Robbelen 1986b). This indicates that parts of the homoeologous chromosomes still possess sufficient affinity to allow pairing with other pairs of homologues. This recombination could add to the variability observed in the amphidiploids.

The study of chromosome pairing in interspecific hybrids helps deduce homology between the contributing genomes. *B. oleracea* × *B. nigra* hybrids are reported to show a low level of pairing with more than 13 chromosomes left unpaired (Attia and Robbelen 1986a). In the present study, the reciprocal cross showed 4–11 univalents in most of the cells. The higher degree of chromosome pairing observed in the BC hybrid obtained in this

study indicates that the affinity between the B and C genomes varies with genotype and reciprocal cross, and may be higher than so far reported (Attia et al. 1987).

At least three characters, namely the number of pods on the main raceme and the number of primary and secondary branches are known to contribute to high rape yield (Campbell and Kondra 1978). The synthesized *B. carinata* showed a range of desirable attributes, such as early flowering, large number of primary and secondary branches, long pod-bearing branches and high pod density. This variability generated by de novo synthesis can be utilized in crosses with natural *B. carinata* to reconstruct plant types suitable for specific environments, as has been done in the case of *B. napus* (Namai et al. 1980).

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