

seed production. For in vitro culture, ovaries were excised 4 to 6 days after pollination, surface-sterilized, and cultured on the medium of Monnier (1973) supplemented with 10% sucrose and 0.8% Bacto-agar. The cultures were incubated at 25°C, under a 16-hour photoperiod (50-90 $\mu\text{Em}^{-2}\text{s}^{-1}$ fluorescent illumination). The seeds were removed from the cultured ovaries when they had become brown (after 6-7 weeks of culture).

Seeds obtained from siliques maturing in situ or ovaries cultured in vitro were surface-sterilized and germinated on the medium of Murashige and Skoog (1962) supplemented with 2% sucrose and 0.8% Bacto-agar. The seedlings were transferred to soil-less growing mix and raised in the greenhouse.

Chromosome doubling of the hybrid plants was performed with colchicine on rooted cuttings of the plants. The morphology of the hybrid plants and of the allotetraploids was examined. The length of pedicels and siliques and the number of seeds per silique were determined in the parental species and the induced allotetraploids.

For meiotic observations, immature flower buds were fixed in Carnoy's fluid II (6:3:1 95% ethanol:chloroform: glacial acetic acid) for 24 hours. The anthers were excised and squashed in 2% acetocarmine. Twenty pollen mother cells were counted per plant. For pollen viability studies, the anthers were excised from fully opened flowers and squashed in 2% acetocarmine.

RESULTS AND DISCUSSION

Of 96 plants recovered from 3,572 pollinations, six plants were confirmed cytologically and morphologically as hybrids. Five of the hybrid plants were from siliques left on the plant and one plant from an ovary cultured in vitro (Table 1). Overall, the percentage of hybrid plants (i.e. the number of hybrid plants per pollinated flower x 100) averaged 0.2% for siliques left in situ and 0.1% for cultured ovaries. In this study, in vitro culture of ovaries did not improve hybrid seed production.

Pre-fertilization crossing barriers were demonstrated in these crosses by poor pollen tube growth on *B. nigra* stigmas and the accumulation of thick callose plugs in the pollen tubes. Few *B. rapa* pollen tubes were observed in *B. nigra* styler tissue. Post-fertilization barriers were also evident as shown by the high degree of embryo abortion.

The interspecific hybrids and the allotetraploids resembled natural *B. juncea* in morphology. Upper stem leaves were lanceolate and entire and had an extremely short petiole or were sessile, in contrast to the long petiole of the leaves of *B. nigra* and the clasping leaves of *B. rapa*. Lower stem leaves were petioled and lobed, as are those of *B. nigra*. Like *B. nigra*, some of the hybrids had purple pigmentation at the nodes and the lower and upper surfaces of the leaves were more or less hispid-hairy.

The arrangement of the flower buds and the mature flowers and the size and the arrangement of the siliques of the resynthesized *B. juncea* genotypes were intermediate between the diploid parents. The immature flower buds had pedicels of ca. 6-7 mm in length and were positioned at the same level as the opened flowers. The siliques (25-40 mm long) contained 15 to 20 seeds and were borne on pedicels of intermediate length (ca. 10 mm), which formed a 45° angle with the stem, as observed in

natural *B. juncea*.

Table 1. Production of hybrid plants from crosses between *Brassica nigra* strains R890, R1819, R3392, and UC-77-1218, and four cultivars of *B. rapa*

Cross	Siliques maturing in situ			Ovaries cultured in vitro		
	Flowers pollinated	No. of plants	No. of hybrids (%) ¹	Flowers pollinated	No. of plants	No. of hybrids (%)
R890						
x Echo	173	1	0 (0)	24	0	-
x Parkland	268	3	0 (0)	116	8	0 (0)
x R500	102	0	-	0	-	-
x Tobin	157	33	0 (0)	12	0	-
R1819						
x Echo	266	4	1 (0.4)	59	1	0 (0)
x Parkland	253	16	1 (0.4)	114	7	1 (0.9)
x R500	30	0	-	0	-	-
x Tobin	205	2	1 (0.5)	61	0	-
R3392						
x Echo	289	0	-	62	0	-
x Parkland	193	1	0 (0)	38	0	-
x Tobin	236	1	0 (0)	69	0	-
UC-77-1218						
x Echo	247	11	1 (0.4)	42	0	-
x Parkland	200	4	1 (0.5)	12	0	-
x R500	12	0	-	0	-	-
x Tobin	260	4	0 (0)	72	0	-
Total	2,891	80	5 (0.2)	681	16	1 (0.1)

¹(No. of hybrid plants/no. of pollinated flowers) x 100

A feature of the resynthesized genotypes of *B. juncea* with *B. nigra* cytoplasm was their stiff stem, a characteristic not exhibited to the same degree by the allotetraploids with *B. rapa* cytoplasm. In *B. carinata* reciprocal synthetic hybrids, the cytoplasm has been shown to confer characteristics of agronomic value, especially a strong stiff stem and a high harvest index (Prakash and Chopra 1990). The stiff stem character remains to be evaluated under field conditions.

The hybrid plants showed a preponderance of 18 univalents; the number of bivalents was restricted to three as compared to the six to eight bivalents reported by Olsson (1960) and

Prakash (1973b) in hybrids of B. rapa x B. nigra. It may well be that the cytoplasm of B. nigra influences pairing affinities between chromosomes of the A and the B genomes; this is also reflected in the meiotic behavior of induced allopolyploids when chromosomes form only bivalents with occasional univalents. The hybrid plants produced less than 1% stainable pollen, except for one hybrid plant which had 6% stainable pollen.

Meiosis in the colchicine-induced allotetraploids was normal as evidenced by the formation of 18 bivalents at diakinesis and metaphase I in most of the cells observed. The allotetraploids had between 45 and 50% stainable pollen. One of the plants averaged 0.03 seed per pollinated flower upon selfing, while cross-pollination with natural B. juncea gave a seed set of 1.11 seed per pollination.

The genotypes of B. juncea carrying the cytoplasm of B. nigra provide new combinations of cytoplasmic and nuclear genomes, and hence offer new opportunities for furthering the improvement of B. juncea.

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