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Intergeneric (intersubtribe) hybridization between *Moricandia arvensis* and *Brassica* A and B genome species by ovary culture

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Summary. Intergeneric hybrids between *Moricandia arvensis* (C_3–C_4 intermediate species) and *Brassica* A and B genome species (*B. campestris* and *B. nigra*) were produced via ovary culture. When *M. arvensis* was used as a female parent, the hybrid embryo yield (0.25–0.45 embryo per pollination) was similar between two genomes, regardless of the male parent. The reciprocal hybrid using *B. campestris* as a female was also obtained, although yield of embryo was lower (0.02 embryo per pollination). On the other hand, no hybrids were obtained without the in vitro technique. As most hybrid embryos could not develop normal shoots, plants were regenerated by inducing shoots on the cultured hypocotyl. The hybrid nature of the regenerated plant was confirmed morphologically and cytogenetically. A certain amount of bivalents (2.52–2.71) in the hybrids indicated the existence of partial chromosome homology between two genera. The present results indicate that ovary culture is an effective technique for overcoming the crossing barrier between *M. arvensis* and *Brassica* cultivated species.

Key words: *Brassica campestris* – *B. nigra* – Intergeneric hybridization – *Moricandia arvensis* – Ovary culture

Introduction

Wide hybridization is a useful tool to improve cultivated crops for gene transfer and to produce new species and alloplasmic strains. It can also be used to investigate species relationships. In the crop *Brassica*, a number of interspecific and -generic hybridization have been reported (summarized by Harberd and McArthur 1980; Prakash and Hinata 1980). However, as speculated by Harberd (1976), the taxonomic group that is capable of hybridizing with *Brassica* crops is apparently limited to genera of the subtribe Brassicinae and *Raphanus* of the subtribe Raphaninae.

Moricandia arvensis, classified in subtribe Moricandiiae, is reported as a C_3–C_4 intermediate species (Apel et al. 1978; Holady et al. 1981) and seems to be a useful source to introduce low photo-respiration character into *Brassica* crops. Recently, intergeneric hybrids between this species and *Brassica* crops were produced by a conventional crossing method (Apel et al. 1984), a somatic hybridization (Toriyama et al. 1987), and an ovary culture (Takahata 1990). However, all of these hybrids were derived from only the combination of *M. arvensis* and the C genome species of *Brassica*.

In the present study, we report the production and characterization of the hybrids of *M. arvensis* with *B. campestris* (A genome) and *B. nigra* (B genome) through ovary culture.

Materials and methods

The plant material consisted of *M. arvensis* (L.) DC. (n=14, genome Ma), strain 4, obtained from Prof. K. Hinata at the Tohoku University, Japan; *B. campestris* L. (n=10, A) ssp. *parachinensis* cv Saishin and ssp. *rapiferae* cv Kanamachi-kokabu; and *B. nigra* (L.) Koch (n=8, B), strain Ni-116. *M. arvensis* was reciprocally crossed with three strains of *Brassica* species by the ordinary method. Some flowers were used for ovary culture described below, and others were left until siliqua maturity.

The ovaries were excised 3–7 days after pollination. The procedures for sterilization of ovary were carried out as described previously (Takahata 1990). The ovaries were placed on agar-solidified MS medium (Murashige and Skoog 1962) supplemented with and without 500 mg/l casein hydrolysate. The hybrid embryos that developed in ovaries were transferred to growth regulator-free MS or B5 (Gamborg et al. 1968) medium

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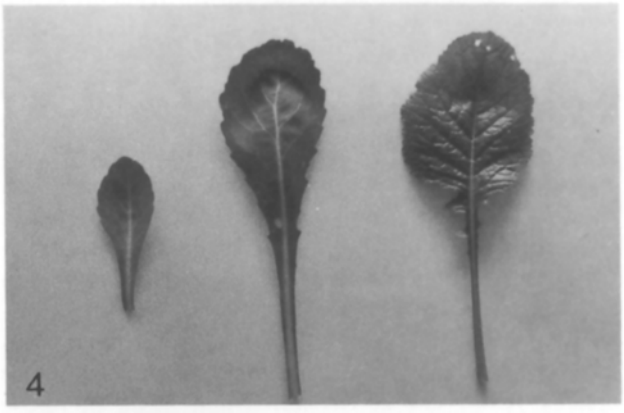
Figs. 1 and 2. Production of intergeneric hybrids between *M. arvensis* and *B. campestris* cv Saishin by ovary culture. **1** Emergence of hybrid embryos from the ovary of *M. arvensis* crossing with *B. campestris* cv Saishin. **2** Regeneration of hybrid plant from the hypocotyl of the hybrid embryo

after 3–4 weeks. As most embryos failed to develop directly into plantlets, we regenerated plants indirectly from hypocotyls as described previously (Takahata 1990). All cultures were grown at 25 °C with 16-h day photoperiod of fluorescent light.

The hybrid nature of regenerated plants was determined morphologically and cytologically. Cytological analysis of mitosis in root-tip cells that were stained with Feulgen solution and of meiosis in pollen mother cells stained with acetocarmine was performed according to Takahata (1990).

Results

After 2–3 weeks, some embryos were detected emerging through the ruptured walls of the ovary, which had turned brown (Fig. 1). Various stage embryos, from torpedo to cotyledon stage embryos, were observed in the ovaries after 4 weeks in culture. In the ovaries of *M. arvensis* crossed with *B. campestris* cv Saishin, 67% of the embryos were cotyledon staged. The number of embryos per ovary varied from 0.45 in *M. arvensis* × *B. campestris* cv Saishin to 0 in *B. campestris* cv Kanamachi-kokabu × *M. arvensis* (Table 1). When *M. arvensis* was used as a female parent, there was no difference in embryo productivity between the crossing of *M. arvensis* with *B. campestris* and that with *B. nigra*. However, a difference in embryo productivity was observed in the reciprocal crosses. That is, the 0.39 embryo production per pollination in *M. arvensis* × *B. campestris* was higher than 0.02 embryo production in the reciprocal cross. A difference in the embryo production was not observed between two media with and without casein hydrolysate. On the other hand, crossability barriers were present in all crosses without the use of the in vitro technique. We obtained no hybrids in 93 crosses of *M. arvensis* × *B. campestris*, in



Figs. 3–5. Morphology of *M. arvensis*, hybrid, and *B. campestris* cv Kanamachi-kokabu (left to right). **3** Young plants. **4** Leaf. **5** Flower

Table 1. Embryo production from ovary culture in hybridizations between *M. arvensis* and *Brassica* A and B genome species

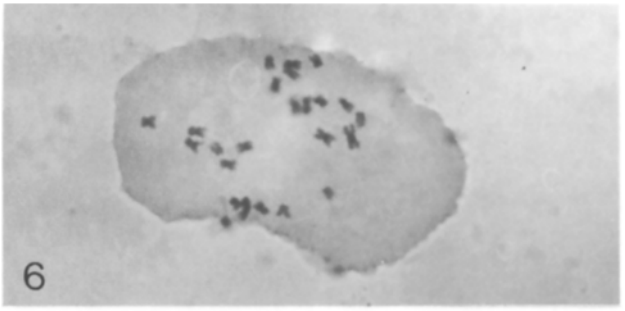
Crosses	No. of cultured ovaries (A)	No. of ovaries inducing embryos	No. of embryos produced (B)	B/A
M. arvensis × *B. campestris*				
cv Saishin	80	13	36	0.45
cv Kanamachi-kokabu	43	6	12	0.28
M. arvensis × *B. nigra*	32	4	8	0.25
B. campestris × *M. arvensis*				
cv Saishin	60	2	3	0.05
cv Kanamachi-kokabu	90	0	0	0.00

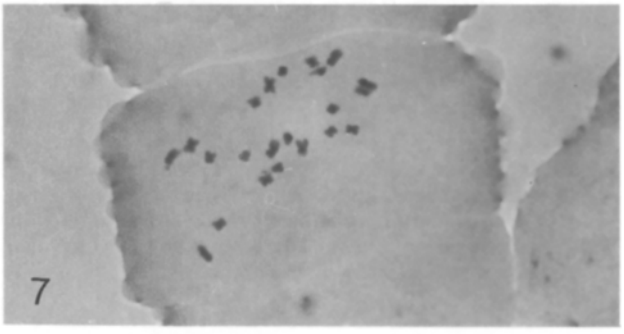
224 crosses of *B. campestris* × *M. arvensis,* or in 61 crosses of *M. arvensis* × *B. nigra.*

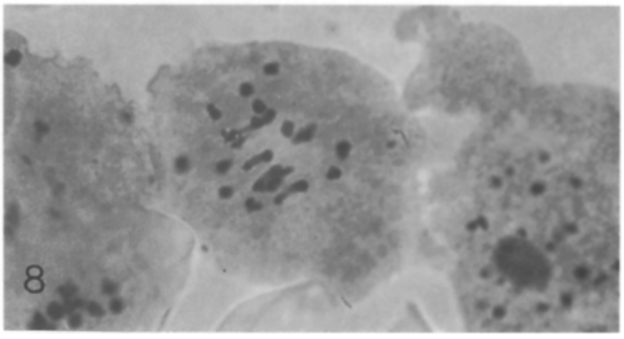
When the embryos were transferred to growth regulator-free MS and B5 medium, most of them proliferated abnormally and did not lead to regenerated plantlets. The same phenomenon was observed with the hybrid embryos of *M. arvensis* × *B. oleracea* (Takahata 1990). Therefore, the regenerated plants were obtained through the induction of shoots in the hypocotyl explants after Takahata (1990). Adventitious shoots were induced from the hypocotyl section on MS medium supplemented with 1.0 mg/l 6-benzyladenine or no growth regulators, and then they were rooted on growth regulator-free MS medium (Fig. 2).

All hybrid plants had morphologically intermediate traits between their parents and were vigorous in their growth. The leaf and flower of the parents were distinct enough to be diagnostic for hybrids. The leaves of *M. arvensis* were fleshy and glaucous, while those of *B. campestris* and *B. nigra* were thin, bright green, and petiolate. Furthermore, *B. nigra* had cleft leaves compared to entire ones of *M. arvensis.* All hybrids showed intermediate characters (Figs. 3 and 4). The flower of the hybrids exhibited combined traits of the parents. The petals were creamy yellow like *B. campestris* and *B. nigra,* whose petals are yellow, but with a purple vein coming from *M. arvensis,* which has purple petal (Fig. 5). The morphological differences were not observed in reciprocal hybrids between *M. arvensis* and *B. campestris* cv Saishin.

Somatic chromosome numbers of hybrids were determined in 9 plants of *M. arvensis* × *B. campestris* cv Saishin, 6 plants of *M. arvensis* × *B. campestris* cv Kanamachi-kokabu, 3 plants of *B. campestris* cv Saishin × *M. arvensis,*and 3 of *M. arvensis* × *B. nigra.* All F_1 plants had the expected chromosome number of 2n = 24 in the hybrids between *M. arvensis* and *B. campestris,* and of 2n = 22 in those of *M. arvensis* × *B. nigra* (Figs. 6 and 7).







Figs. 6–8. Cytological analysis of the hybrids. **6** Somatic chromosomes of the hybrid of *M. arvensis* × *B. campestri* cv Saishin (2n = 24). **7** Somatic chromosomes of the hybrid of *M. arvensis* × *B. nigra* (2n = 22). **8** Meiotic chromosomes of the hybrid of *M. arvensis* × *B. campestris* cv Kanamachi-kokabu (6II + 12I)

Table 2. Chromosome pairing at MI in the hybrids between *M. arvensis* and *Brassica* A and B genome species

Crosses	No. of plants observed	2n	No. of PMCs observed	Chromosome conjugation		
				I	II	III
M. arvensis × *B. campestris* cv Saishin	3	24	75	18.51 (24–12)	2.71 (6–0)	0.03 (1–0)
cv Kanamachi-kokabu	4	24	100	18.71 (24–12)	2.63 (6–0)	0.01 (1–0)
M. arvensis × *B. nigra*	1	22	25	16.72 (22–12)	2.52 (5–0)	0.08 (1–0)
B. campestris × *M. arvensis* cv Saishin	1	24	25	18.80 (24–14)	2.60 (5–0)	0.00

The data on chromosome associations at Metaphase I in the four hybrids are given in Table 2. Meiotic irregularities were observed in all hybrids, but they exhibited a certain amount of bivalent formation (Fig. 8). In hybrids of *M. arvensis* × *B. campestris*, the average and maximum values of bivalents were 2.63–2.71 and 6, respectively. A similar frequency of chromosome paring was also observed in the reciprocal cross. The hybrid of *M. arvensis* × *B. nigra* exhibited 2.52 and 5 on average and a maximum value of bivalents, respectively. These were similar values as those of the hybrids between *M. arvensis* and *B. campestris*. Almost all hybrids exhibited shriveled anthers and had no fertile pollen.

Discussion

It is speculated that the taxonomic group that is able to exchange genes with *Brassica* crops by sexual hybridization is consistent with subtribe Brassicinae and some genera of Raphaninae (Harberd 1976). As far as is known, all hybrids that were produced by sexual and somatic hybridization agree with this speculation, except for the hybrids with *Arabidopsis thaliana* (Gleba and Hoffmann 1980) and *Moricandia arvensis* (Apel et al. 1984; Toriyama et al. 1987; Takahata 1990).

The present paper describes the production of new intergeneric hybrids between *M. arvensis* and *Brassica* A and B genome species through ovary culture. Until now, the hybrids between *M. arvensis* and *Brassica* C genome species were obtained by sexual crossing (Apel et al. 1984) and somatic hybridization (Toriyama et al. 1987). Recently, Takahata (1990) reported that it was possible to produce many hybrid embryos from these crosses via ovary culture. The embryo productivity in this study (0.25–0.45 embryos per pollination) was lower than the result obtained using *B. oleracea* as a parent (2.71) (Takahata 1990). Apel et al. (1984) also obtained hybrids between *B. alboglabra* (C genome) and *M. arvensis* by conventional crossing methods. These results indicate that *M. arvensis* has a weaker cross-incompatibility with the C genome than with the A and B genomes. On the other hand, the reciprocal hybrids between *M. arvensis* and *B. campestris* were obtained in this study. Takahata (1990) failed to produce the hybrids in ovary culture when *B. oleracea* was used as a female. The easier recovery of the hybrids from ovary culture using *B. campestris* as a female parent rather than using *B. oleracea* agree with the results of the hybridizations between *Brassica* cultivated species (Inomata 1978; Takeshita et al. 1980; Matsuzawa 1983).

In the production of alloplasmic lines through wide hybridization, Ringdahl et al. (1987) indicated that it is difficult to obtain intergeneric hybrids between wild species and *Brassica* crops, and they speculated that the embryo culture technique is also very difficult because of the small seed of wild specis. Ovary culture has been used to produce interspecific hybrids between *Brassica* cultivated species (Inomata 1977; Matsuzawa 1978; Mohapatra and Bajaj 1988), and Nanda Kumar et al. (1988) obtained interspecific hybrids between *B. fruticulosa* and *B. campestris* through ovary culture. This technique is easier than embryo and ovule culture, because there is no need to remove embryo and ovule. We have recently obtained several intergeneric and -specific hybrids by this technique (Y. Takahata, T. Funada, T. Takeda unpublished results). These results suggest that ovary culture is an easy and effective tool for wide hybridization in the crucifers.

Although *M. arvensis* is classified in the subtribe Moricandiieae and is distantly related with *Brassica* in Brassicinae by conventional taxonomy (Schulz 1936), a certain amount of bivalents were observed in the PMCs of the hybrids. The range and mean frequency of bivalents in the present hybrids were similar to those of hybrids between *M. arvensis* and C genome species (Takahata 1990). The existence of the partial chromosome homology between *Moricandia* and *Brassica* indicates that the phylogenetic relationship between them must be closer than that based on conventional taxonomy.

M. arvensis, which is reported to be C_3–C_4 intermediate species, has useful agronomic traits such as a low photo-respiration activity. Recently, some other species in *Moricandia* were also reported to be C_3–C_4 intermediate species (Hylton et al. 1988). The results in this study indicate that there is the possibility for introduction of this useful trait into *Brassica* crops.

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