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N. Inomata

Intergeneric hybridization between *Brassica napus* and *Sinapis pubescens*, and the cytology and crossability of their progenies

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Abstract The cytological possibility of gene transfer from *Sinapis pubescens* to *Brassica napus* was investigated. Intergeneric hybrids between *Brassica napus* (2n = 38) and *Sinapis pubescens* (2n = 18) were produced through ovary culture. The F_1 hybrids were dihaploid and the chromosome configurations were $(0–1)_{III} + (2–11)_{II} + (5–24)_I$. One F_2 plant with 38 chromosomes was obtained from open pollination of the F_1 hybrid. Thirty-one seeds were obtained from the backcross of the F_2 plant with *B. napus*. Five out of seven plants had 38 chromosomes, and the pollen stainability ranged from 0% to 81.4%. In the B_2 plants obtained from the backcross of B_1 plants with *B. napus*, 66.7% of the plants examined had 38 chromosomes. *S. pubescens* may become a gene source for the improvement of *B. napus*.

Key words *Brassica napus* · Crossability · Cytogenetics · Intergeneric hybridization · *Sinapis pubescens*

Introduction

In crop improvement it is necessary to expand gene sources by the selective introgression of alien genes into a good agronomic base. Wide hybridization can be used to transfer the desirable variability. The subtribe *Brassicinae* consists of five genera, *Brassica*, *Diplotasix*, *Eruca*, *Erucastrum* and *Sinapis* (Mizushima 1952), and there are many wild species among these. The genus *Brassica*, for example, comprises 159 species, a number of which are wild species of economic importance (Bajaj et al. 1986). Both interspecific and intergeneric hybridization have a great potential in the breeding of cruciferous crops despite the fact that it is difficult to produce such hybrids due to cross-incompatibility barriers. Recent developments in biotechnology and embryo rescue techniques may provide new genetic variability for the breeding of cruciferous crops (Chiang et al. 1978, 1980; McNaughton and Ross 1978; Mohapatra and Bajaj 1987; Inomata 1990, 1992, 1993a). However, little information on the progeny of the F_1 hybrid is available.

The present paper reports the intergeneric hybridization between *Brassica napus* and *Sinapis pubescens* by means of ovary culture. The cytology and crossability of the progenies were investigated. The possibility of gene transfer from *S. pubescens* to *B. napus* is discussed. A preliminary report of the experiment presented here was made in a previous paper (Inomata 1991b).

Materials and methods

The plants used in the experiment were *Brassica napus* ssp. *oleifera* cv 'Aomori No. 1' (2n = 38) and *Sinapis pubescens* (2n = 18). The *B. napus* used in the backcross was the same as that used in the production of the F_1 hybrid. When emasculated flowers bloomed, reciprocal crosses were carried out by the conventional method. Ovary culture was carried out as described previously (Inomata 1985a, 1990). The F_2 and F_3 plants were obtained from open pollination of the F_1 and F_2 plants, respectively. The B_1 plants were obtained from the backcross of the F_2 plant with *B. napus*.

Stainability of the pollen, pollen fertility and the chromosome configuration at the PMCs were examined by acetic carmine. Chromosome numbers of the root tips were checked as described previously (Inomata 1977).

Results

Production of F_1 hybrids

Table 1 shows the results of intergeneric hybridization between *B. napus* and *S. pubescens*. Nine seeds were harvested from this cross, five of which germinated. Two F_1 hybrids grew. In a reciprocal cross, one developing embryo that attained the late torpedo-shaped stage was obtained, but it did not grew further. The F_1 hybrids were of a type intermediate between the parents in

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N. Inomata
Department of Biology, College of Liberal Arts and Sciences, Okayama University, Okayama 700, Japan

Table 1 Intergeneric hybridization between *Brassica napus* and *Sinapis pubescens* through ovary culture

Cross combination	Number of capsules examined	Number of embryos further cultured	Number of seeds obtained	Number of hybrids obtained
B. napus × *S. pubescens*	103	0	9	5
Reciprocal cross	57	1[a]	0	0

[a] Developmental stage of the embryo was the late torpedo-shaped stage

general morphological characters (Fig. 1B) and had dihaploid chromosome numbers.

Cytology, pollen fertility and crossability of the F_1 hybrid

The chromosome configuration at the first meiotic division was counted in 80 cells of the two F_1 hybrids. The mode of chromosome configuration was $10_{II} + 8_I$ followed by $11_{II} + 6_I$ and $7_{II} + 14_I$ (Fig. 3A), and reached 76.5%. Chromosome configurations showed $(0–1)_{III} + (2–11)_{II} + (5–24)_I$.

The F_1 hybrids were completely pollen sterile in 1000 pollen grains examined (Fig. 2A). The abortive pollen grains were of various shapes, i.e. large-sized, tetrad-type (Fig. 2B) and dyad-type (Fig. 2C). In the initial stages of pollen development nucleus division occurred but cell division did not follow, and four and two nuclei remained in one cytoplasm (Fig. 2D, E). Dyads and monads in microspore mother cells were obtained in the developmental stage (Fig. 2F). In the meiotic stage of pollen development, the nucleus did not divide synchronously (Fig. 2G). Table 2 shows the frequency of different shapes of division in microspore mother cells: over one-half of the microspore mother cells were dyads, with tetrads being the next most frequent form.

No seeds were obtained from self-pollination (89 flowers), nor from the backcross with *B. napus* (1392 flowers). Six seeds were harvested from open pollination of the 260 flowers examined.

Morphology, cytology and crossability of the F_2 plant

Six seeds were planted and one F_2 plant grew. This plant had 38 chromosomes, and the morphology of the leaf chracteristics was more similar to that of *B. napus* than was the F_1 hybrid. At the beginning of inflorescence, the flower structure was normal but the petals did not open completely. The pistils in the small buds at the upper part of the inflorescence appeared before anthesis, but the flowers did not develop further.

The mode of chromosome configuration at the PMCs was $15_{II} + 8_I$ followed by $1_{III} + 12_{II} + 11_I$ (Fig. 3B), $16_{II} + 6_I$ and $13_{II} + 12_I$, and reached 73.3% in the 30 cells observed. Chromosome configurations at the PMCs showed $(0–1)_{IV} + (0–2)_{III} + (11–17)_{II} + (4–12)_I$. Pollen fertility was 0.8% in the 622 pollen grains that were counted. As the development of microsporogenesis was poor, anthers did not develop well. The morphology of the sterile pollen grains was similar to that shown by their counterparts of the F_1 hybrid.

In the pollination experiment, the flowers were only at the beginning of inflorencence. Two seeds were obtained from 18 flowers by open pollination, and 31 seeds were obtained from 582 flowers upon backcross of the F_2 plant with *B. napus*.

Morphology, cytology and crossability of the F_3 and B_1 plants, and their progenies

Two plants derived from open pollination grew, and 10 seeds from the backcross were planted, 5 of which grew. The morphology of the leaves resembled that of *B. napus* more than that of the F_2 plant. The chromosome num-

Fig. 1A–C Intergeneric hybrid between *Brassica napus* and *Sinapis pubescens*, and their parents. **A** *B. napus* ssp. *oleifera* cv 'Aomori No. 1' was used as the female plant, **B** F_1 hybrid between *B. napus* and *S. pubescens* with 28 chromosomes, **C** *S. pubescens* was used as the male plant

Table 2 Frequency of different shapes of division in microspore mother cells in the F_1 hybrid between *Brassica napus* and *Sinapis pubescens*

Different shapes of division in microspore mother cell				
Monad	Dyad	Triad	Tetrad	Total
42	386	0	172	600

bers ranged from 38 to 47. The mode of chromosome configuration at the PMCs was $18_{II} + 2_I$ followed by $17_{II} + 4_I$ and $16_{II} + 6_I$, and reached 87.1%. Chromosome configuration at the PMCs showed $(0–1)_{IV} + (0–1)_{III} + (15–19)_{II} + (2–6)_I$.

Table 3 shows chromosome number, pollen fertility and crossability of the F_3 and B_1 plants. The chromosome numbers of F_4 and B_2 plants were investigated in some of their progenies. All F_3 and B_1 plants were normal for inflorescence and anthesis, but pollen fertility ranged from 0% to 81.4%. Microspore mother cell development was examined in three plants, and the most frequent shape was the tetrad. Many seeds were har-

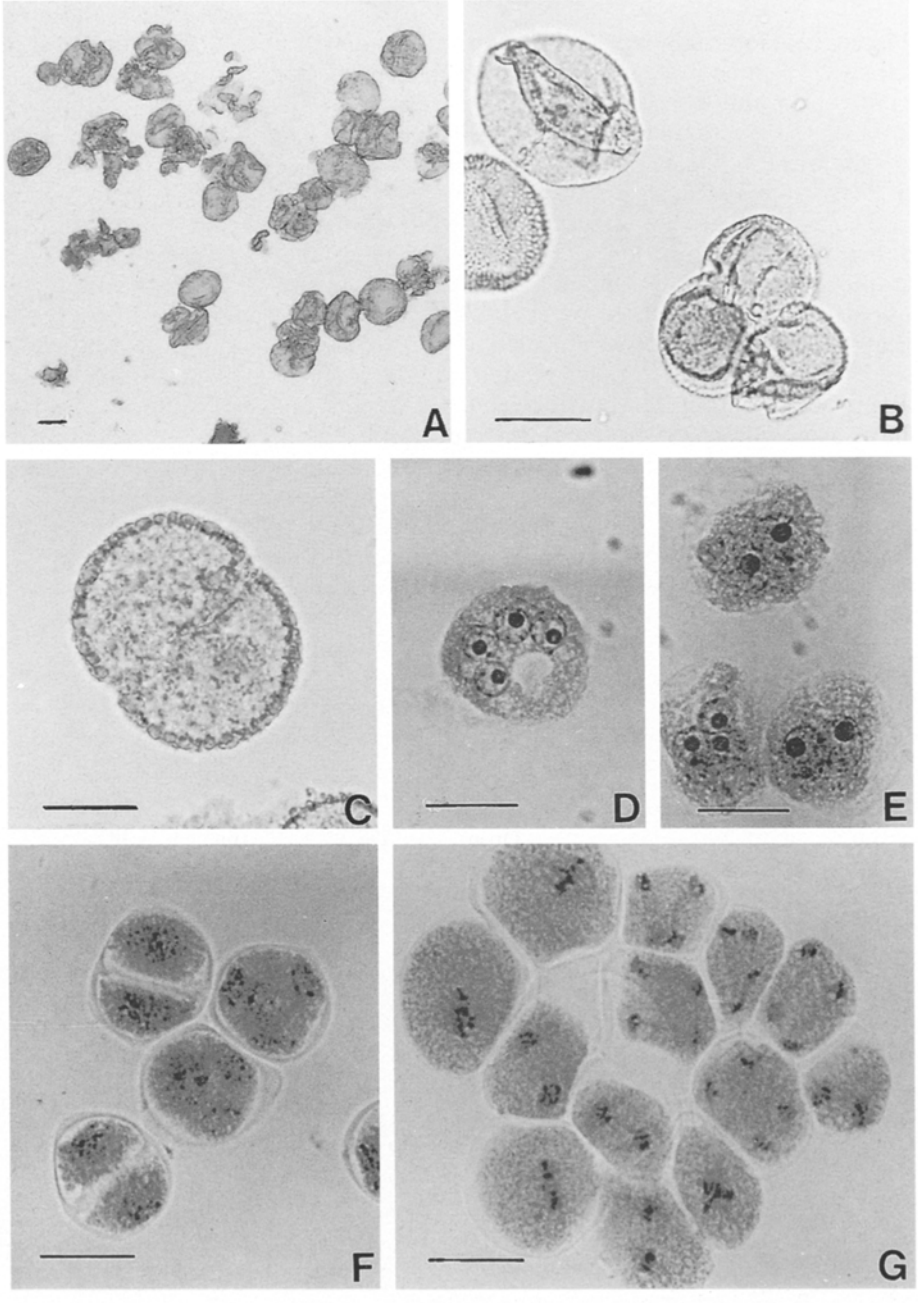

Fig. 2A–G Pollen fertility and pollen development of the F_1 hybrid between *B. napus* and *S. pubescens*. **A** No pollen fertility, **B** abortive pollen grains that were interrupted at the second meiotic division of pollen mother cell and pollen tetrad, **C** pollen development was interrupted at the first meiotic division, **D** microspore with four nuclei, **E** microspores with two and four nuclei, **F** dyads and monads in microspore mother cell after first and second meiotic division, **G** asynchronous division of meiosis. *Bar*: 20 μm

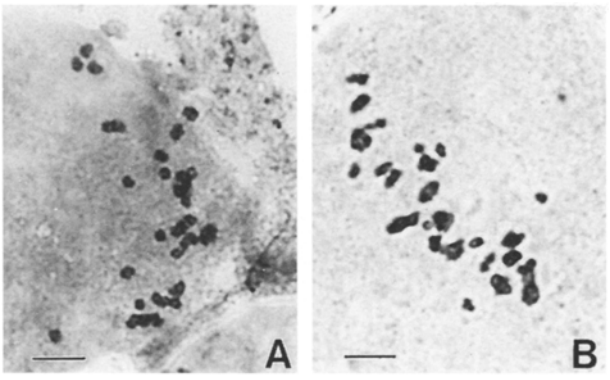

Fig. 3A,B Meiotic chromosomes in the PMCs of F_1 and F_2 plants. **A** $7_{II} + 14_I$ in F_1 hybrid, **B** $1_{III} + 12_{II} + 11_I$ in F_2 plant. Bar: 5 μm

vested from F_2 and B_1 plants with 38 chromosomes. The most frequent progenies of their plants had 38 chromosomes, and aneuploid plants having from 27 to 56 chromosomes were examined. In B_1 plants with 44 chromosomes, the most frequent progeneies from open pollination and B_2 plants had 38 chromosomes. No seeds were obtained from the F_3 plant with 47 chromosomes.

Discussion

By means of the embryo rescue technique, investigators have been able to obtain many interspecific and intergeneric hybrids between *Brassica* crops, and between *Brassica* crops and wild relatives (Inomata 1990, 1992, 1993b; Mathias 1991; Yadav et al. 1991; Gundimeda et al. 1992; Nanda Kumar and Shivanna 1993). In the present investigation ovary culture was effective in producing intergeneric hybrids between *B. napus* and *S. pubescens*.

With respect to chromosome configuration in the PMCs from interspecific and intergeneric hybridization in *Brassiceae*, some bivalent formation has been observed in the crosses of *B. juncea* × *Sinapis pubescens* (Inomata 1991b) and *Diplotaxis siettiana* × *B. campestris* (Nanda Kumar and Shivanna 1993). On the other hand, a high incidence of bivalent formation was observed in the crosses of *B. campestris* × *B. oleracea* (Inomata 1980; Attia and Röbbelen 1986), *B. campestris* × wild relatives of *B. oleracea* (Inomata 1985b, 1986, 1987), *B. napus* × *Sinapis pubescens* (Harberd and McArthur 1980), *Moricandia arvensis* × *Brassica* A-, B- and C-genome species (Takahata 1990; Takahata and Takeda 1990), *B. carinata* × *Sinapis turgida* (Inomata 1992) and *Sinapis alba* × *B. napus* (Lelivelt et al. 1993). In the present experiment, the F_1 hybrids were dihaploid. Though they consisted of three separate genome constitutions, good bivalent formation was observed. The genome of *S. pubescens* may have the homology of the A and/or C-genome of *B. napus*.

The size of the normal pollen grains was not uniform and different shapes were observed in dividing microspore mother cells of an interspecific hybrid between *B. campestris* and *B. oleracea* (Inomata 1980); different shapes of microspore mother cell and microspore were also observed in an intergeneric hybrid between *B. carinata* and *S. turgida* (Inomata 1992). In the present experiment, various shapes and sizes of abortive pollen

Table 3 Chromosome number, pollen fertility and crossability of the F_3 and B_1 plants derived from the open pollination of F_2 plants and backcross of F_2 plants with *Brassica napus* respectively, and chromosome number in some of their progenies

Number of plants observed	Chromosome number (2n)	Pollen fertility	Type of pollination of F_3 and B_1 plants	Progeny				
		Range and mean (%)[a]		Number of flowers pollinated	Number of seeds obtained	Number of seeds sown	Number of seeds germinated	Chromosome number in root-tip cell (2n)[b]
5[c]	38	1.0–81.4 38.1	Self-pollination	139	288	115	111	35(1), 36(2), 37(4), 38(56), 39(1), 40(11), 41(1), ?(35)
			Open pollination	277	1162	183	172	27(1), 29(1), 31(1), 32(1), 34(1), 36(2), 37(30), 38(83), 39(12), 40(8), 41(3), 42(4), 47(1), 56(1), ?(23)
			B. napus	97	597	148	141	31, 36&39(1), 32, 35&38(1), 34(2), 36(4), 37(14), 38(86), 38,&40(1), 39(9), 40(7), 41(1), 42(1), ?(12)
1	44	0.6	Self-pollination	0	–	–		
			Open pollination	45	34	34	29	32(1), 35(1), 37(1), 38(9), 39(1), 40(4), 41(2), 42(5), 44(1), 45(1), ?(3)
			B. napus	21	33	33	33	36(1), 38(17), 40(2), 41(1), 42(5), 44(1), 45(1), ?(5)

[a] From 500 to 1777 pollen grains were counted
[b] Number in parentheses is the number of plants observed and ? indicates that the chromosome number could not be determined
[c] All plants which had 38 chromosomes were included

grains appeared, and development might have been interrupted between the first meiotic division and pollen tetrad.

In the progeny of the interspecific hybrid between *B. campestris* and *B. oleracea*, it might be possible to introduce the genes of *B. campestris and B. oleracea* to *B. napus* (Inomata 1983), and effect a reciprocal exchange (Inomata 1991a). In the progeny of the intergeneric hybrid between *B. carinata* and *Sinapis turgida*, the genes of *S. turgida* can be introduced to *B. carinata* (Inomata 1992). With respect to hybrid progenies of the cross between *B. campestris* and three wild relatives of *B. oleracea*, *B. bourgeaui*, *B. cretica* and *B. montana*, gene transfer might be possible from the wild relatives of *B. oleracea* to *B. campestris* and *B. napus* (Inomata 1993b). In the present experiment, the majority of the progenies from F_3 and B_1 plants had 38 chromosomes. It may be possible that the genes of *S. pubescens* can be introduced to *B. napus*. The embryo rescue technique may be effective in gene transfer from wild relatives of cruciferous plants to *Brassica* crops.

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