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Intergeneric hybridization of *Diplotaxis erucoides* with *Brassica napus*. I. Cytogenetic analysis of F_1 and BC_1 progeny

R. Delourme, F. Eber and A.M. Chevre
I.N.R.A. Station d'Amélioration des Plantes, Domaine de la Motte au Vicomte, B.P. 29, 35650 Le Rheu, France

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Summary

Intergeneric hybrids (F_1) *Diplotaxis erucoides* (DeDe) × *Brassica napus* (AACC) and the first backcross to *B. napus* (BC_1) have been obtained through in vitro culture of excised ovaries. The chromosome numbers of F_1 and BC_1 plants proved the occurrence of unreduced gametes. The study of metaphase I chromosome pairing showed that autosyndesis in De genome and allosyndesis between De and A/C genomes might exist. The male fertility of the F_1 plants was low. Some male-sterile plants were found in F_1 and BC_1 progeny. The possibilities of creating addition lines *B. napus – D. erucoides* and of obtaining a new cytoplasmic male sterility in *B. napus* are discussed.

Introduction

Interspecific and intergeneric hybridization have been widely used in crop breeding. In many instances, rapeseed (*Brassica napus* L., genome AACC, 2n = 38) has been crossed to other *Brassica* species and to related genera (Prakash & Hinata, 1980).

The use of addition lines to study genome structure is well demonstrated in the *Triticineae* (Law, 1981). Studies aiming to develop these cytogenetic stocks in *Brassica* are just beginning (Fantes & MacKay, 1979; Quiros et al., 1985, 1986; Jahier et al., 1987). The production of disomic addition lines in which single pairs of homologous chromosomes from *D. erucoides* (L.) DC are added to rapeseed would be of great interest; *D. erucoides* is one of the species with the lowest chromosome number known (2n = 14) in the *Brassiceae*. It has already been successfully crossed to *B. napus* (Harberd & McArthur, 1980; Ringdahl et al., 1987).

Moreover, another *Diplotaxis* species, *D. muralis* (L.) DC, was successfully crossed, as female parent, with *B. campestris* L., which produced a cytoplasmic male sterility (CMS) in *B. campestris* (Hinata & Konno, 1979). This CMS was then transferred to *B. napus* (Pellan-Delourme & Renard, 1987).

This study reports the hybridization of *D. erucoides* and *B. napus* and examines (i) the cytogenetic relationships between rapeseed and *D. erucoides* genomes (ii) the possibility of obtaining a new CMS in rapeseed.

Materials and methods

An accession of the wild species, *Diplotaxis erucoides,* collected near Saragossa (Spain), by G. Pelletier was kindly supplied to us. The *B. napus*

line used was a spring oilseed line 'RP_1'. For the hybridizations, the plants were grown in the greenhouse during the spring of 1985. *D. erucoides* was used as the female parent. The buds were emasculated, pollinated with rapeseed pollen and bagged. The ovaries were excised 4 days after pollination, surface sterilized twice with calcium hypochloride (70 g/l) for 15 minutes, rinsed twice in sterile distilled water and cultured in Petri dishes. The composition of the E_{12} medium used is given in Table 1 (Rousselle & Eber, in press). Three to five ovaries were placed in each Petri dish. The cultures were incubated in a growth chamber at 20° C day/15° C night and 300–500 lux of light for 16 hours a day. The embryo-derived plantlets were transplanted into pots in the greenhouse as soon as they developed roots. When the plants had 5–6 leaves, they were placed in a cold chamber (at 4° C) and kept there until they were transplanted in the greenhouse in the spring of 1986. Ovary culture was also necessary to obtain the first backcross (BC_1) to *B. napus,* using the latter as the pollen parent. Two *B. napus* lines were used for these backcrosses: 'RP_1' and 'Darmor', a winter oilseed line.

Table 1. Composition of the E_{12} medium

Components	Concentrations in mg/l
$Ca(NO_3)_2, 4H_2O$	584.0
$MgSO_4, 7H_2O$	431.5
$(NH_4)_2, SO_4$	58.0
KH_2PO_4	136.0
KCl	361.0
NH_4NO_3	334.0
H_3BO_3	6.200
$MnSO_4, H_2O$	16.700
$ZnSO_4, 7H_2O$	10.700
$Na_2MoO_4, 2H_2O$	0.250
$CuSO_4, 5H_2O$	0.025
$CoCl_2, 6H_2O$	0.025
$FeSO_4, 7H_2O$	27.800
Na_2 EDTA	37.300
KI	0.830
Inositol	100.0
Pyridoxine	0.5
Thiamine	0.5
Caseine hydrolysate	300.0
Glutamine	600.0
Nicotinic acid	1.5
Sucrose	15,000
Glucose	15,000
Agar	8,000

Chromosome counts were made on root meristems 2 or 3 days after transplanting the plants into pots. Roots were treated with a solution of 8-hydroxyquinoline (0.29 g/l) for 4 hours at room temperature and fixed with (1 : 3) acetic-ethanol for at least 30 minutes at 4° C. Then they were hydrolysed in 1N HCl for 10 minutes at 60° C and coloured with Schiff's reagent (Jahier et al., 1987).

For chromosome observations in pollen mother cells (PMCs), floral buds were fixed in Carnoy's solution (ethanol, chloroform, acetic acid – 6 : 3 : 1, respectively) with chloral (0.05%) for 48 hours. PMCs were examined at the metaphase I meiotic stage by squashing anthers in a drop of aceto-carmine (Jahier et al., 1987).

Male fertility of the hybrids was estimated by pollen grain stainability in aceto-carmine. For each plant, three flowers were removed and at least 500 pollen grains were counted per flower. Male fertility of the BC_1 plants was visually assessed.

Results

Production of F_1 hybrids and successive backcrosses

Sixty-six F_1 plants (*D. erucoides* × *B. napus*) (Fig. 1) were grown from 432 ovaries cultured i.e. 15.3 plants per 100 ovaries. The flowers of the hybrids exhibited the white flower colour derived from *D. erucoides* but some of them were cream or white with yellow sectors. F_1 plants were crossed with 2 rapeseed lines to get BC_1 progeny. The number of ovaries cultured and number of plantlets obtained are shown in Table 2. There was no significant difference between the two lines for the number of plantlets obtained per 100 ovaries cultured (t test; $p>0.05$). Backcrosses on 45 F_1 plants only resulted in 244 BC_1 plantlets i.e. 4.8 plantlets per 100 ovaries cultured. Seventy-five BC_1 plants were grown in the greenhouse. Most of them had cream or pale

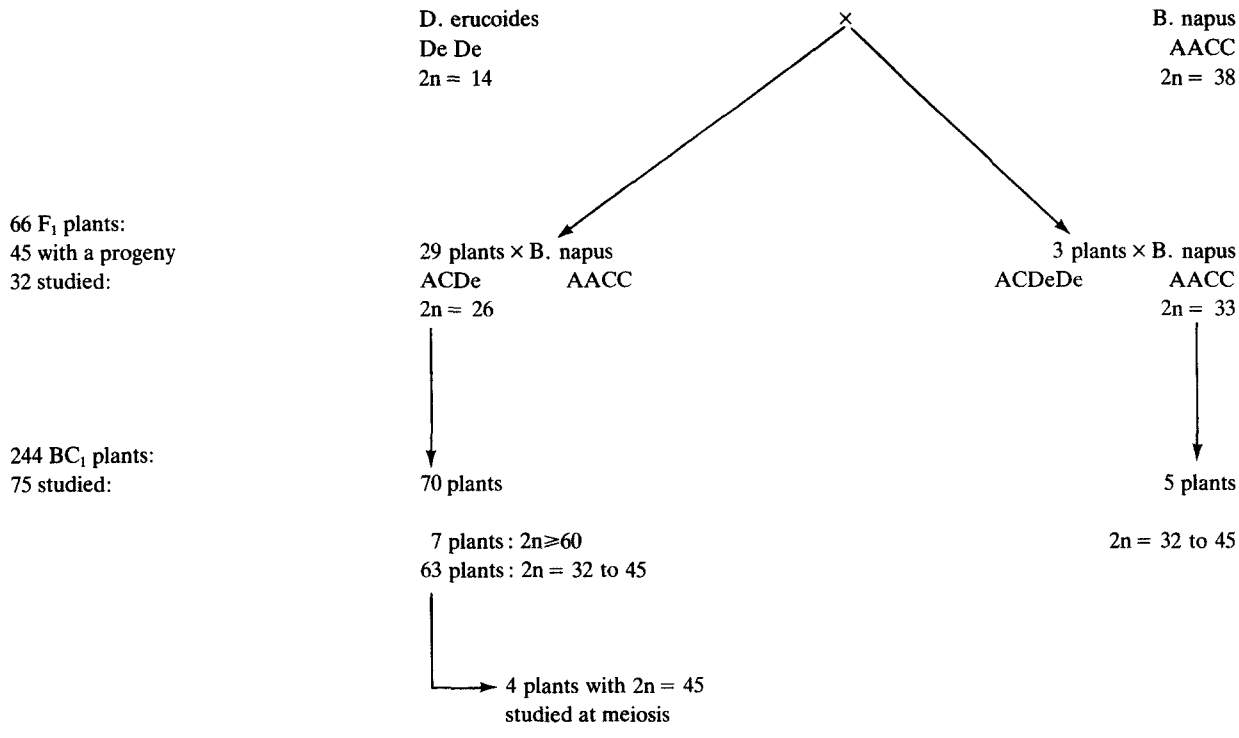

Fig. 1. Genealogy and chromosome number of F_1 and BC_1 plants.

yellow flowers with a distinct yellow center. A few plants exhibited the white flower colour of *D. erucoides* or the yellow colour of *B. napus*. Seeds of the second backcross (BC_2) to *B. napus* could be obtained on 25 out of the 75 BC_1 plants without using in vitro culture.

Cytogenetic studies

Among the 32 F_1 plants analysed, 29 plants had the expected number of 26 chromosomes and 3 plants had 33 chromosomes (Fig. 1). The meiotic behaviour of the plants with the same chromosome number was not significantly different (Table 3).

The chromosome number of 75 BC_1 plants was analysed (Fig. 1). Seventy BC_1 plants were obtained from the F_1 plants with 2n = 26. Their chromosome number ranged from 32 to 45 except for seven plants which had more than 60 chromosomes. Five BC_1 plants obtained from the F_1 plants with 2n = 33 had also 32 to 45 chromosomes. The meiotic behaviour of the parental line 'RP_1' and of 4 BC_1 plants with 45 chromosomes (coming from 4

Table 2. Production of BC_1 progeny by in vitro culture of excised ovaries of *D. erucoides* × *B. napus* hybrids pollinated by two rapeseed lines

Rapeseed line	Number of female F_1 plants	Number of ovaries cultured (A)	Number of plantlets obtained (B)	Number of plantlets per 100 ovaries (B/A × 100)
RP_1	61	3928	181	4.6
DARMOR	38	1166	63	5.4
Total	61	5094	244	4.8

F_1 plants with 26 chromosomes) is shown in Table 4. The meiotic behaviour of these 4 plants was compared using analysis of variance. There was no significant difference between the plants for the mean number of univalents ($p>0.05$) but a highly significant plant effect was found for the mean number of bivalents ($p<0.01$) and multivalents ($p<0.01$).

Male fertility

Pollen stainability of 41 F_1 plants was studied. It ranged from 3.0 to 37.1% with an average of 15.2%. Besides, two plants were completely male-sterile. Among the 75 BC_1 plants examined, 13 plants were completely male-sterile and 8 were partially male-sterile. Partially male-sterile plants were male-sterile at the beginning of the flowering period but produced some pollen later. One of the male-sterile F_1 plants gave one male-sterile and two partially male-sterile BC_1 plants. The other one had no progeny.

Discussion

Hybrids between *D. erucoides* and *B. napus* are difficult to obtain without using in vitro culture. Ringdahl et al. (1987) reported only 3 hybrid plants from 561 pollinations. Since embryos aborted too early, embryo culture could not be used and ovary culture was attempted. This allowed the production of more hybrid plants compared to the results of Ringdahl et al. (1987). The yield can probably be improved when ovaries are dissected and devel-

Table 3. Metaphase I chromosome pairing (± SE) of the F_1 hybrids between *D. erucoides* and *B. napus*

Number of chromosomes	Number of PMCs	Chromosome pairing per PMC			
		Univalents	Bivalents	Trivalents	Quadrivalents
26	933	12.96 ± 0.81 (4–20)[1]	6.13 ± 0.39 (3–10)	0.14 ± 0.07 (0–2)	0.09 ± 0.04 (0–1)
33	97	11.85 ± 0.74 (6–23)	10.30 ± 0.33 (5–13)	0.09 ± 0.04 (0–1)	0.07 ± 0.07 (0–1)

[1] range.

Table 4. Metaphase I chromosome pairing (± SE) of the *B. napus* parental line 'RP_1' and of 4 BC_1 plants (*D. erucoides* × *B. napus*[2])

Code	Number of chromosomes	Number of PMCs	Chromosome pairing per PMC			
			Univalents	Bivalents	Trivalents	Quadrivalents
RP_1	38	50	0.12 ± 0.48 (0–2)[1]	18.94 ± 0.24 (18–19)	0	0
BC_1-1	45	17	5.35 ± 2.39 (2–11)	16.35 ± 1.73 (13–20)	0.59 ± 0.71 (0–2)	1.29 ± 0.59 (0–2)
BC_1-2	45	28	6.39 ± 2.61 (2–11)	16.32 ± 1.02 (15–19)	0.75 ± 0.84 (0–3)	0.93 ± 0.60 (0–2)
BC_1-3	45	10	7.70 ± 2.49 (4–11)	15.30 ± 2.00 (12–19)	0.50 ± 0.53 (0–1)	1.30 ± 0.67 (0–2)
BC_1-4	45	25	7.00 ± 1.91 (3–11)	18.12 ± 1.51 (15–21)	0	0.44 ± 0.65 (0–2)

[1] range.

oping embryos are subcultured instead of letting them grow inside the ovaries. Generally BC_1 progeny are easier to obtain than F_1 interspecific or intergeneric hybrids. However in this study, the use of ovary culture was again necessary to get BC_1 progeny and the number of plantlets produced for 100 ovaries was lower than for F_1 hybrids production. Only BC_2 progeny could be obtained without in vitro culture.

F_1 hybrid plants had either 26 or 33 chromosomes. The plants with 26 chromosomes most likely have the expected ACDe genomic constitution while those with 33 chromosomes probably are ACDeDe. The latter might originate from the fertilization of unreduced egg-cells of *D. erucoides*. This phenomenon is known to occur in the *Brassiceae* with a relatively high frequency (Heyn, 1977; Jahier et al., 1987). The plants with 33 chromosomes showed on average 10.3 bivalents at the metaphase I meiotic stage (Table 3). On account of preferential pairing between homologous genomes, the two De genomes must form 7 bivalents. Then the remaining 3.3 bivalents and the multivalents should result from autosyndesis in A or C genomes and/or from allosyndesis between A and C genomes. This corresponds with the amount of pairing observed in AC haploids (Renard & Dosba, 1980; Attia & Röbbelen, 1986). The plants with 26 chromosomes showed on average 6.13 bivalents. This is in agreement with the observations of Harberd & McArthur (1980) who found 7.55 bivalents (with a range from 3 to 10) in hybrids ($2n = 26$) between *B. napus* and *D. erucoides*. Assuming that pairing in AC genomes is the same in ACDe plants as in ACDeDe plants, 3 bivalents would result from autosyndesis in De genome or/and from allosyndesis between De and A/C genomes. According to Mizushima (1972), up to 4 allosyndetic bivalents can be formed between De and C genomes. The percentage of chromosome paired (50%) in ACDe plants is similar to that of ACB plants (*B. napus* × *B. nigra* hybrids) (Busso et al., 1987) or to that of ACR plants (*B. napus* × *Raphanus sativus* hybrids (Rousselle, 1983).

The F_1 plants with 26 chromosomes produced BC_1 plants with 32 to more than 60 chromosomes. Thus, the F_1 hybrids produced reduced, unbalanced (plants with $2n = 32$ to 44) or unreduced egg-cells (plants with $2n = 45$). The presence of plants with more than 60 chromosomes shows that gains in chromosome number occur in these hybrids, as reported in the progeny of hybrids between *B. oleracea* and *Raphanus sativus* (Cauderon, pers. comm.). This might result from the fertilization of unreduced egg-cells by unreduced pollen grains or of double unreduced egg-cells by normal pollen grains (Heyn, 1977). The meiotic behaviour of 4 plants with 45 chromosomes was analysed (Table 4) since they were likely to have the AACCDe genomic constitution. In 11% of the pollen mother cells (PMCs), the number of univalents decreased down to 3 or 2. This indicate that at least 4 or 5 chromosomes of De genome can pair together or with A/C genomes. In 4 PMCs out of 80, 20 bivalents and 5 univalents were observed. This might indicate that autosyndesis can occur in De genome. Allosyndesis De-A and/or De-C must no be ruled out because of the near invariable presence of at least one quadrivalent. However, the levels of metaphase I pairing of the 4 BC_1 plants with 45 chromosomes were significantly different as far as numbers of bivalents and multivalents were concerned. The plant BC_1-4 showed a more regular meiotic behaviour than the others (Table 4). The possibility of intergenomic recombination in the F_1 plants cannot be discounted, despite the non-reduction of female gametes. It could explain the differences in pairing behaviour displayed by the BC_1 plants. Only the study of BC_2 plants will help to draw more solid conclusions.

The BC_2 plants with 19 bivalents and univalents will be selected and backcrossed with rapeseed to obtain monosomic addition lines. After selfing, it might be possible to breed stable disomic addition lines. Their study and their comparison with addition lines *B. napus* – *B. nigra* (Jahier et al., 1987) will provide information on the genomic structure and the phylogeny of the *Brassiceae*. At the same time, recombination between the genomes of *D. erucoides* and *B. napus* might be detected. To complete these data, biochemical and molecular markers will be used.

Furthermore, two male-sterile F_1 plants and thirteen male-sterile BC_1 plants were observed. The

sterility in these plants could only be due to chromosome abnormalities. In order to test the cytoplasm of *D. erucoides* for male sterility, the plants with 45 chromosomes will be backcrossed to rapeseed to eliminate De chromosomes and obtain the alloplasmic line with 38 rapeseed chromosomes. Ringdahl et al. (1987) found that *D. erucoides* does not possess an effective male sterility-inducing-cytoplasm in conjunction with 3 genotypes of rapeseed. The transfer of *Diplotaxis muralis* cytoplasmic male sterility from *B. campestris* to *B. napus* has shown that only 2 out of 147 rapeseed lines were maintainer lines and allowed the expression of male-sterility (Pellan-Delourme & Renard, 1987). If the frequency of maintainer lines is as low for *D. erucoides* as for *D. muralis* then a great number of genotypes has to be tested before being able to detect the effect of *D. erucoides* cytoplasm on male sterility in *B. napus*.

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