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Intergeneric hybridization between *Diplotaxis siifolia*, a wild species and crop brassicas

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Summary. Attempts were made to obtain intergeneric hybrids between *Diplotaxis siifolia*, a wild species, and cultivars of *Brassica* (*B. campestris, B. juncea,* and *B. napus*). The crosses showed unilateral incompatibility. When the wild species was used as female parent, pollen germination and pollen tube growth were normal, but hybrid seeds aborted due to post-fertilization barriers. Reciprocal crosses (cultivars as female parent) showed strong pre-fertilization barriers; although pollen grains showed germination, pollen tubes failed to enter the stigma. Hybrids were realized in two of the crosses, *D. siifolia* × *B. juncea* and *D. siifolia* × *B. napus*, through ovary culture. The hybrids were multiplied in vitro by multiplication of axillary shoots, or somatic embryogenesis. Detailed studies were carried out on the hybrid *D. siifolia* × *B. juncea.* F_1 hybrids had shrivelled anthers and were pollen sterile. Amphiploids of this hybrid showed 60% pollen fertility and produced seeds upon self-pollination as well as backcross pollination with the pollen of *B. juncea*.

Key words: *Brassica campestris – B. juncea – B. napus – Diplotaxis siifolia* –Intergeneric hybrid

Introduction

The genus *Brassica* comprises some of the most important oil-yielding species. Most of the cultivars are susceptible to white rust and alternaria blight. Wild species of *Brassica* are potential sources of genes conferring resistance to these diseases. The wild species have also been identified as sources for cytoplasmic male sterility (Ogura 1968; Hinata and Konno 1979; Prakash and Chopra 1988), needed for exploiting hybrid vigor.

The genus *Diplotaxis* has 20 diverse species endemic to the Mediterranean region. *Diplotaxis siifolia,* used in the present investigation, is resistant to white rust and alternaria blight. It has been suggested that some genetic homology exists between *Diplotaxis* species and crop brassicas (Gómez-Campo and Tortosa 1974), which would allow introgression of genes from the wild species to the cultivars.

Many successful reports of wide hybrids using crop brassicas have appeared in recent years (Takeshita et al. 1980; Nanda Kumar et al. 1988a, b, 1989; Agnihotri et al. 1988; Batra et al. 1989). Earlier attempts to cross *D. siifolia* with *B. napus* were not successful (Ringdahl et al. 1987). The present study reports details of pollen germination and pollen tube growth in intergeneric crosses between *D. siifolia* and crop brassicas (*B. campestris, B. juncea* and *B. napus*), realization of hybrids through embryo rescue in two of the crosses (*D. siifolia* × *B. juncea* and *D. siifolia* × *B. napus*), and details of the hybrids and amphiploids of the cross *D. siifolia* × *B. juncea.*

Materials and methods

Plants of *Diplotaxis siifolia* G. Kunze ($n=10$, Ds), *Brassica campestris* L. ($n=10$, A) ssp. *oleifera* var. brown sarson cv Pusa Kalyani, *B. juncea* (L.) Czern. ($n=18$, AB), and *B. napus* L. ($n=19$, AC) ssp. *oleifera* strain 706, were grown under field conditions. Pollinations were carried out on emasculated flowers on the day of anthesis. Pollinated flowers were rebagged and left on the plant until maturity/senescence or used for ovary culture. Pollen germination and pollen tube growth were studied through aniline blue fluorescence of pollinated pistils.

To culture ovaries, pistils were excised 4–7 days after pollination and cultured on White's medium containing 5% sucrose,

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0.7% agar, and 500 mg/l casein hydrolysate, and were maintained under continuous illumination (23 W/m^2) at 25° ± 2 °C. The seeds were harvested after the ovaries turned brown.

Seeds obtained from both ovary cultures and field pollinations were sown directly in soil or on MS medium containing 2% sucrose and 0.7% agar. F_1 hybrids were multiplied in vitro through culture of shoot-tip and nodal segments on MS medium containing 0.5 mg/l 6-benzylaminopurine (BAP). Shoots were rooted on MS medium containing 0.5% agar and 0.1 mg/l α-napthaleneacetic acid (NAA) and were transferred to pots containing soil and grown to flowering.

To induce amphiploidy, an aqueous solution of colchicine (0.1%, 0.2%) was applied with small cotton plugs to the axillary meristems of single-node segments cultured on MS medium containing 0.5 mg/l BAP. Colchiploid shoots, produced in vitro, were rooted and subsequently transferred to the soil.

For cytological studies, flower buds of F_1 and amphiploids were fixed in Carnoy's solution and squashed in 2% acetocarmine.

Results

Field pollinations did not yield hybrid plants in any of the combinations (Table 1). When *D. siifolia* was used as the female parent, a few shrivelled seeds were obtained in the cross *D. siifolia* × *B. napus*. However, none of them germinated. All the combinations in which the cultivars were used as female parent gave rise to some seeds. In the cross *B. juncea* × *D. siifolia*, most of the seeds were shrivelled; only seven of them that were healthy germinated, and all of them turned out to be matromorphs. Also in the cross *B. napus* × *D. siifolia* all the seedlings turned out to be matromorphs.

Studies on pollen germination and pollen tube growth following intergeneric crosses showed unilateral incompatibility. The pistil of the wild species, *D. siifolia*, permitted profuse pollen germination and pollen tube growth of all the cultivars (Fig. 1 A, C). The pistils of the cultivars, although they allowed germination of *D. siifolia* pollen, did not permit entry of pollen tubes into the papillae (Fig. 1 B). Callose deposition was observed in pollen tubes as well as on the tips of the papillae.

Intraspecific pollinations of the pistils of *D. siifolia* showed it to be a partially self-incompatible species. The number of germinated pollen grains and pollen tubes in self-pollinated pistils was markedly less than that observed in cross-pollinated pistils. Even the number of seeds developed in selfed flowers was significantly less than that in crossed flowers. The index of self-incompatibility (Zapata and Arroyo 1978) was 0.2, characteristic of partially self-incompatible species.

Table 2 presents the responses of cultured ovaries. Although mostly shrivelled seeds were obtained through ovary culture in all the combinations using *D. siifolia* as the female parent, a few of them did germinate and gave rise to hybrid seedlings in the crosses *D. siifolia* × *B. juncea* and *D. siifolia* × *B. napus*. In the reciprocal crosses some seeds were realized only in the cross *B. napus* × *D. siifolia*, but all of them turned out to be matromorphs.

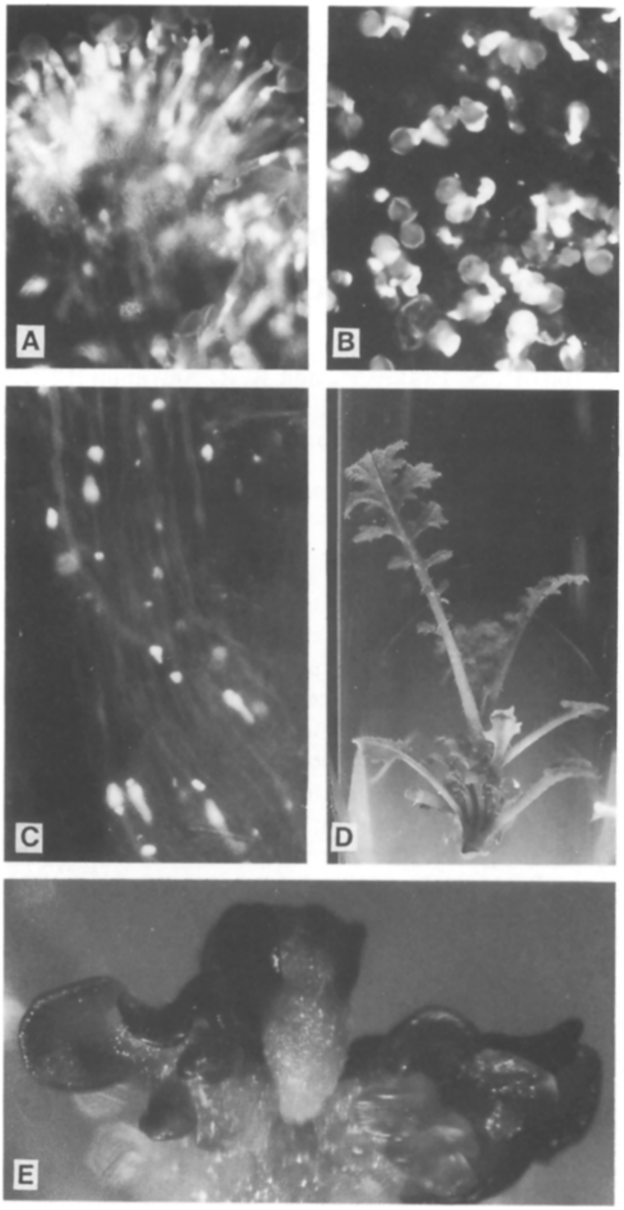

Fig. 1 A–E. Intergeneric hybridization between *Diplotaxis siifolia* and crop brassicas. **A, C** Fluorescent micrographs of the stigma (**A**) and style (**C**) of *D. siifolia* pollinated with pollen of *B. juncea* to show pollen germination and pollen tube growth. **B** As in A, but of the stigma of *B. campestris* pollinated with pollen of *D. siifolia*; although pollen grains have germinated, pollen tubes have not entered the stigma. **D** Shoot tip of the hybrid *D. siifolia* × *B. juncea* cultured for shoot multiplication. **E** Somatic embryos produced from cotyledons of the hybrids *D. siifolia* × *B. napus*

The hybrid seedlings obtained in the cross *D. siifolia* × *B. juncea* were multiplied through in vitro culture of nodal segments and shoot tips (Fig. 1 D). Many amphiploids were also realized through the in vitro applica-

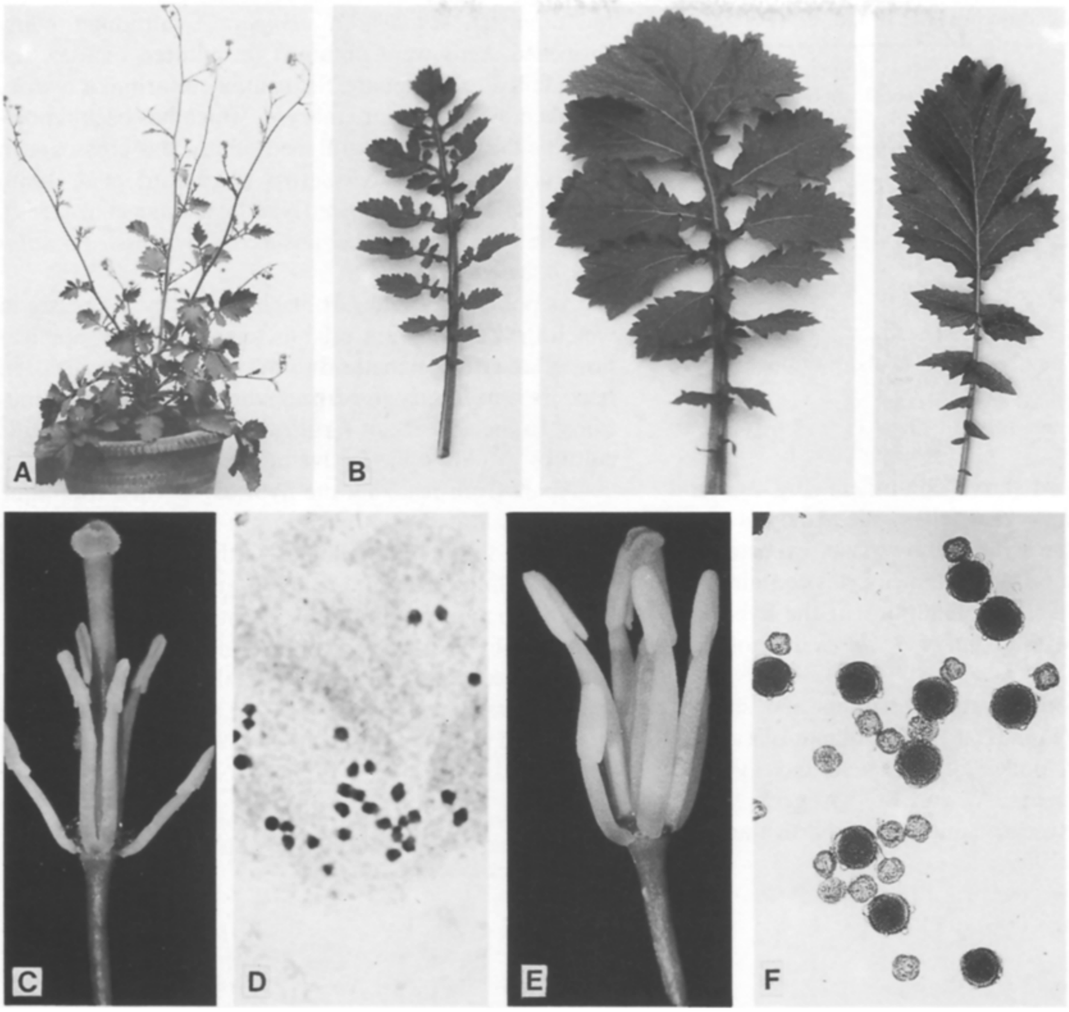

Fig. 2A–F. Morphology and cytology of the hybrid *D. siifolia* × *B. juncea* and its amphiploid. **A** F_1 hybrid grown to flowering. **B** Lower leaves (from *left* to *right*) of *D. siifolia*, the hybrid and *B. juncea*. **C** The stamens and the pistil of the hybrid; the anthers are shrivelled. **D** Meiotic metaphase I of the hybrid showing 28 univalents. **E** The stamens and pistil of the amphiploid stained with acetocarmine to show fertile and sterile pollen

Table 1. Responses of field pollinations of intergeneric crosses

Cross	No. of pollinations	No. of fruits formed	No. of seeds	No. of hybrid seedlings	No. of matromorphs
D. siifolia × *B. campestris*	101	46	0	0	0
D. siifolia × *B. juncea*	63	36	0	0	0
D. siifolia × *B. napus*	78	28	9	0	0
B. campestris × *D. siifolia*	88	56	1	0	0
B. juncea × *D. siifolia*	53	19	68	0	7
B. napus × *D. siifolia*	74	35	7	0	7

tion of colchicine to the axillary meristem. In-vitro-grown plants were transferred to soil and 25 F_1 hybrids (Fig. 2A) and 10 amphiploids were grown to flowering.

The other hybrid, *D. siifolia* × *B. napus*, produced somatic embryos and shoot buds from the cotyledons (Fig. 1E). The details of these plants will be studied during the next season.

Morphology and cytology of D. siifolia × B. juncea hybrid and amphiploid

Hybrids largely resembled the male parent, particularly in general morphology and inflorescence. In a few characters such as leaf dissection (Fig. 2B) and petal color, the hybrids were intermediate between the parents.

Table 2. Responses of cultured ovaries of intergeneric crosses

Cross	No. of ovaries cultured	No. of seeds	No. of hybrid seedlings	No. of matromorphs
D. siifolia × *B. campestris*	54	20	0	0
D. siifolia × *B. juncea*	35	13	3[a]	0
D. siifolia × *B. napus*	73	3	2[a]	0
B. campestris × *D. siifolia*	56	0	0	0
B. juncea × *D. siifolia*	18	0	0	0
B. napus × *D. siifolia*	61	7	0	7

[a] Used to raise a large number of hybrid plants through in vitro multiplication

F_1 plants produced shrivelled anthers (Fig. 2C) and the pollen grains were completely sterile. Analysis of the M_1 of meiosis of the hybrid showed, as expected, 28 chromosomes. A majority of the meiocytes contained 28 univalents (Fig. 2D). Self-pollinations of the hybrid as well as backcrosses with pollen of *B. juncea* did not yield any seeds.

Amphiploids produced turgid anthers and showed 60% pollen fertility (Fig. 2E, F). Self-pollinations as well as backcrosses with pollen of *B. juncea* have yielded many A_2 and BC_1 seeds. A_2 and BC_1 progeny will be grown during the next season and studied particularly for cytoplasmic male sterility.

Discussion

The crosses between the wild species, *D. siifolia*, and the cultivars showed typical, unilateral incompatibility as far as pollen germination and pollen tube growth are concerned. Pollen grains of all three cultivars showed good pollen germination on the stigma and pollen tube growth in the pistil of the *D. siifolia*. Although germinated on stigmas of the cultivars, pollen tubes from pollen grains of *D. siifolia* failed to enter the stigma of any of the cultivars tested. Thus, crosses in which cultivars were used as female parents showed strong pre-fertilization barriers, while pre-fertilization barriers did not operate in reciprocal crosses. Such unilateral incompatibility is reported in many other crosses of *Brassica* (Harberd 1976).

Strong post-fertilization barriers operate even in crosses involving *D. siifolia* as female parent, as revealed by the failure to realize hybrid seeds through field pollinations. As has been reported in many other crosses of *Brassica* (Harberd and McArthur 1980; Nanda Kumar et al. 1989; Agnihotri et al. 1988; Batra et al. 1989), embryo rescue was inevitable for realization of the hybrids. Ovary culture was successful in raising hybrids in two of the crosses attempted – *D. siifolia* × *B. juncea* and *D. siifolia* × *B. napus*. Ovary culture was not effective in the cross *D. siifolia* × *B. campestris;* although many shrivelled seeds were obtained in cultured ovaries, the seeds failed to germinate. Sequential culturing of ovaries and then ovules and/or embryos, which has been reported to be the only successful method in a few crosses such as *Eruca sativa* × *B. campestris* (Agnihotri et al. 1988) and *B. napus* × *B. gravinae* (Nanda Kumar et al. 1989), may be effective in the unsuccessful cross, *D. siifolia* × *B. campestris.*

As pollen tubes do not reach the ovary in crosses in which the cultivars are used as female parents, application of an effective method to overcome pre-fertilization barriers is necessary to realize hybrids in these combinations. In the absence of fertilization, ovary and/or ovule cultures would be ineffective in obtaining the hybrids.

Apart from realizing the hybrids, in vitro technique greatly facilitated multiplication of the hybrids and induction of fertile amphiploids. As the number of hybrid seeds/seedlings realized through ovary culture were limited, in vitro multiplication was essential for detailed studies on the hybrids and for induction of colchiploids. A_2 and BC_1 seeds were successfully realized in amphiploids of the cross *D. siifolia* × *B. juncea,* and these would be grown and studied during the next season.

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