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DIPLOTAXIS TENUISILIQUA × *BRASSICA CAMPESTRIS* HYBRIDS OBTAINED BY EMBRYO RESCUE

B. K. SARMAH* AND N. SARLA

Division of Genetics
Indian Agricultural Research Institute, New Delhi 110 012

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ABSTRACT

Studies on pollen germination, pollen tube growth and micropylar penetration in the cross *D. tenuisiliqua* × *B. campestris* revealed that 0.06% ovules were fertilized at 5 days after pollination. Field pollination did not set seeds in this cross. Sequential culture of ovaries and ovules helped obtain intergeneric hybrids. Starch gel electrophoresis for peroxidases established true nature of the hybrids.

Key words : Intergeneric hybrids, embryo rescue, *Diplotaxis tenuisiliqua, Brassica campestris*

Intergeneric hybridization between the related wild and cultivated *Brassica* sps. offers tremendous scope for the improvement of crop brassicas, specially for the development of cytoplasmic male sterile (cms) lines. Two species of *Diplotaxis muralis* and *D. siifolia* have provided alloplasmic cms systems in *Brassica* [1-3]. However, we need diverse cms systems in crop brassicas to avoid disease risk in monoculturing. With this in view *D. tenuisiliqua* used as female parent was crossed to *B. campestris* to obtain intergeneric hybrid which is the first step towards the development of alloplasmic cms systems in *B. campestris*.

MATERIALS AND METHODS

Pistils of *D. tenuisiliqua* were pollinated with *B. campestris* pollen. Forty pollinated pistils were studied for pollen germination, pollen tube growth and micropylar penetration at 5 days after pollination (dap) using Aniline Blue Fluorescence method [4]. Another set of 200 pollinated pistils were left on the plant upto maturity to observe seed set. For sequential ovary-ovule culture, 65 ovaries were cultured at 10 dap in MS medium +400 mg/l casein hydrolysate (CH) [MS_1]. One pistil was placed

*Present address : Department of Plant Breeding and Genetics, Assam Agricultural University, Jorhat, Assam 785 013

in one culture tube. Tubes were numbered as DC1 to DC65. After 15-20 days when ovaries turned brown, ovules were dissected out and subcultured in MS medium + 5% sucrose +400 mg/l CH + 1 mg/l Kn (MS_2). Cultures were kept in dark till the embryos grew out. Calli developed at hypocotyl of germinated seeds were subcultured in MS medium +0.5 mg/l BAP + 0.1 mg/l NAA (MS_3) for regeneration to take place. For the maintenance/multiplication of the hybrids excised shoot tips were cultured in MS medium + 0.5 mg/l BAP (MS_4). Cultures were kept in continuous light (3000-4000 lux) at 25 ± 2°C. Hybrid shoots were rooted in MS medium + 0.1 mg/l NAA (MS_5).

Horizontal starch gel electrophoresis was used to study peroxidase isozyme pattern in young leaves of parents and hybrids. The relative migration (Rm) of each band was calculated as :

$$R_m = \frac{\text{Distance of band from origin}}{\text{Total distance run}}$$

RESULTS AND DISCUSSION

Fluorescence microscopic studies of the pistils at 5 dap showed good pollen germination (Fig. 1). 0.6% ovules showed pollen tubes at their micropyles. Thus, fertilization took place in this cross. To study if embryos developed, ovules from 10 pistils (20 dap) were dissected and stained with 2% acetocarmine. Most of the ovules were degenerated and did not take the stain. However, one globular embryo was seen in a degenerated ovule. On the other hand 15 dap old selfed pistils of *D. tenuisiliqua* had turgid ovules, 80% of which contained heart shaped embryos. Slower growth of embryos in the cross indicated their hybrid nature.

Only 10 siliquae were obtained from 200 pollinated pistils left on the plant upto maturity. These siliquae did not contain any seed (Table 1). Thus field pollination could not give rise to hybrids in this cross.

Sequential culture of ovary-ovule helped obtain hybrids in this cross. From the 65 ovaries cultured in MS_1 medium, in all 232 ovules were transferred to MS_2 medium. Out of these only 4 ovules germinated (after 35-45 days) and later produced callus at the base of the hypocotyl. Percent ovule germination was 1.7% (Table 1). Thus there was a three fold increase in percent ovules with embryo in sequential culture over percent ovule fertilization (0.06%) at 5 dap. Further, in vivo ovules had only globular embryos at 20 dap. Thus sequential ovary-ovule culture helped increase percent ovule fertilization, embryo development and obtain hybrids. Sequential ovary-ovule culture has also been used to obtain intergeneric hybrids between other species of *Diplotaxis* and *Brassica* [5-6]. Seedlings from four germinated ovules in four different culture tubes were designated as hybrids DC1, DC2, DC3 and DC4. The calli developed at hypocotyl were again subcultured in MS_3 medium for their

Table 1. Comparative results of field pollination and sequential culture in the cross *D. tenuisiliqua* × *B. campestris*

Methods to obtain hybrid	No. of pollinations (A)	Seed set/ ovules cultured (B)	Seeds/ovules germinated (C)	%' Seed/ovule germination (hybrid seedling) (B/C × 100)	Efficiency per pollination B/A
Field pollination	200	0	0	0.00	0.00
Sequential ovary-ovule culture	65	232	4	1.70	0.06

regeneration. Shoots with new leaves regenerated (Fig. 2). The shoots did not elongate and a bunch of leaves regenerated from the callus. Leaves were intermediate in shape and had very long petioles.

As the hybrids were obtained in the off season, they were maintained/multiplied *in vitro* by culturing the excised shoot tips in MS_4 medium. All the four hybrids produced callus at the base of the shoots and the callus later showed regeneration of 0- 4 shoots (Fig. 3). Out of the four hybrids only hybrid DC2 could be maintained upto a period of 8 months by continuous subculturing in the same medium. Shoots of the hybrids DC2 were cultured in MS_5 medium for the induction of roots and 25% of cultured shoots produced roots (Fig. 4).

Peroxidase isoenzymes of leaves were studied in hybrid DC2 and the two parents to prove the true nature of the hybrid. *D. tenuisiliqua* had only 1 anodal band (Rm = 0.2) and *B. campestris* had only 1 cathodal band (Rm = 0.44). The hybrid DC2 had 2 bands, one similar to *D. tenuisiliqua* band (Rm = 0.2) and the other similar to *B. campestris* band (Rm = 0.44). The allozymes from male parent are codominantly expressed in a true hybrid[7].

The hybrid DC2 did not flower *in vitro* during its maintenance in MS medium + 0.5 mg/l BAP under continuous light (3000-4000 lux) while two other intergeneric hybrids *Erucastrum abyssinicum* × *Brassica oleracea* and *E. abyssinicum* × *B. juncea* flowered *in vitro*. Cytokinins as well as environmental conditions are known to influence flowering *in vitro* [8]. Several combinations of environment and hormones need to be tested for induction of flowering *in vitro*. This will facilitate *in vitro* back crossing of the hybrid and back crossed progenies can directly be transplanted into the soil [9]. Alloplasmic lines can thus be obtained in a short period of time.

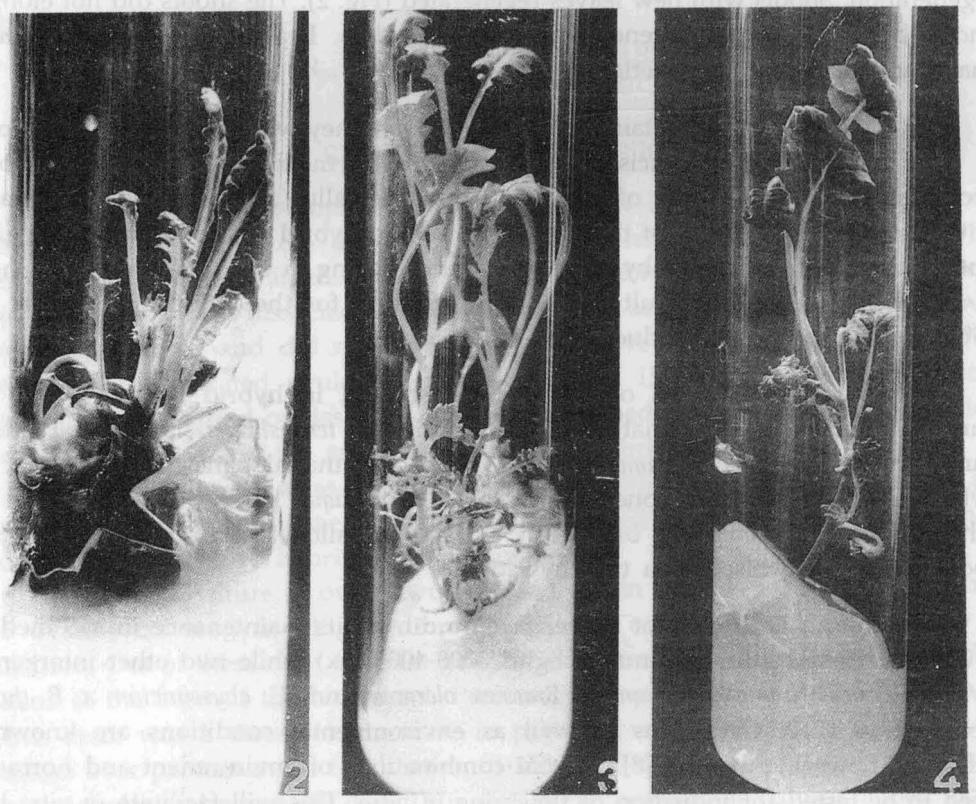

Fig. 1. Aniline Blue stained squash preparations showing pollen tubes of *B. campestris* inside the stigmatic papillae of *D. tenuisiliqua*, viewed under fluorescence microscope
Fig. 2. Shoots with new leaves regenerated from the hypocotyl callus of Hybrid DC2
Fig. 3. Multiple shoots of Hybrid DC_2 regenerated from callus at base of shoot tip
Fig. 4. Hybrid DC_2 in the rooting medium

REFERENCES

1. K. Hinata and N. Konno. 1979. Studies on a male sterile strain having the *Brassica campestris* nucleus and the *Diplotaxis muralis* cytoplasm. Japan J. Breed., **29**: 305- 311.
2. R. P. Delourme and M. Renard. 1987. Identification of maintainer genes in *Brassica napus* L. for the male- sterility inducing cytoplasm of *Diplotaxis muralis* L. Plant Breeding, **99**: 87-89.
3. G. U. Rao, V. Batra-Sarup, S. Prakash and K. R. Shivanna. 1994. Development of a new cytoplasmic male sterile system in *Brassica juncea* through wide hybridization. Plant Breeding, **112**: 171-174.
4. H. F. Linskens and K. Esser. 1957. Uber eine spezifische Anfarbung der pollen schlauche im Griffel und die Zahl der Kallosepropfen nach Selbstung and Fremdung. Naturwissenschaften., **44**: 16.
5. V. Batra, S. Prakash and K. R. Shivanna. 1990. Intergeneric hybridization between *Diplotaxis siifolia*, a wild species and crop brassicas. TAG., **80**: 537-54.
6. P. B. A. Nanda Kumar and K. R. Shivanna. 1993. Intergeneric hybridization between *Diplotaxis siettiana* and crop brassicas for the production of alloplasmic lines. TAG., **85**: 770-776.
7. N. F. Weeden. 1989. Application of isozymes in plant breeding. Plant breeding Reviews., **6**: 11-54.
8. N. J. Roberts, G. A. Luckman and R. C. Menary. 1993. *In vitro* flowering of *Boronia megastigma* Nees. and the effect of 6- Benzylaminopurine. J. Plant Growth Regul., **12**: 117-122.
9. Sarmah, B. K. and N. Sarla. 1997. *In vitro* flowering and back crossing of *Brassica* intergeneric hybrids. Current Science., **72**(10): 702-704.