Broadening the Genetic Base of Crop Brassicas by Production of New Intergeneric Hybrid

Aparajita MOHANTY[1], Babeeta CHRUNGU[2], Nidhi VERMA[3] and Kundaranahalli Ramalingaiah SHIVANNA[4]

[1]Department of Botany, Gargi College, University of Delhi, New Delhi, India; [2]Zakir Husain College, Jawaharlal Nehru Marg, New Delhi, India; [3]National Bureau of Plant Genetic Resources, IARI Campus, New Delhi, India; [4]Ashoka Trust for Research in Ecology and the Environment, Hebbal, Bangalore, India

Abstract: Wide hybridization between crop brassicas and their wild relatives is an important approach towards increasing the genetic variability, which can be utilised for brassica breeding programs. A new intergeneric hybrid between *Erucastrum cardaminoides* and *Brassica oleracea* var. alboglabra was produced using embryo rescue techniques. The F_1 hybrid was intermediate between the male and female parent for most of the morphological characters. Cytological studies of pollen mother cells of the hybrid revealed a preponderance of univalents at metaphase I. The number of bivalents in the digenomic hybrid was lower than expected. However, the presence of trivalent and quadrivalent in cells indicated some homoeology between the two genomes and hence the possibility of introgression of genes into the cultivar. The first backcross progeny was obtained using *B. oleracea* var. alboglabra as the pollen parent. Further, it is being used for developing new alloplasmic lines. The intergeneric hybrid was also used as bridge species to transfer wild (*E. cardaminoides*) cytoplasm to *B. napus* and *B. carinata*. The new intergeneric hybrid and bridge cross hybrids produced in the present investigation have contributed towards increasing the genic and cytoplasmic variability and thus broadening the genetic base of crop brassicas.

Keywords: *Brassica oleracea* var. alboglabra; embryo-rescue; *Erucastrum cardaminoides*; genetic variability; wide hybridization

Generation of genic and cytoplasmic variability is very important for the improvement of crop brassicas through breeding programs. Wild and weedy relatives of crop brassicas are known to be reservoirs of genes that impart resistance to biotic and abiotic stresses (Warwick *et al*. 2000). Moreover, their cytoplasm can induce cytoplasmic male sterility (CMS) in crop brassicas, which is important for hybrid seed production in oilseed brassicas, and heterosis in vegetable brassicas (Sigareva & Earle 1997; Primard-Brisset *et al*. 2005; Bhat *et al*. 2007). The development of alloplasmic lines has also shown important agronomic traits like early flowering and dwarfness in *B. napus* (Rao *et al*.1998). An important approach to effectively utilize the wild germplasm for the development of alloplasmic and CMS lines or to introgress desirable traits is the production of wide hybrids between cultivars and wild relatives followed by repeated backcrosses (Prakash 2001; Bang *et al*. 2007). The wide hybrids also allow the study of intergenomic affinities between the wild

and cultivated species and hence the possibility of introgression of desirable genes into cultivars (Chandra *et al*. 2004; Garg *et al*. 2007; Nicolas *et al*. 2008).

The genus *Erucastrum* (tribe *Brassiceae*) has 23 species but only four species have been utilized for intergeneric hybridization with crop brassicas. *Erucastrum cardaminoides* is a hardy plant that grows on volcanic soils and in rocky fields (Gomez-Campo 1984; Warwick *et al*. 2000). It shows resistance to white rust, downy and powdery mildews and is tolerant to *Alternaria* blight (Gupta *et al*. 1995). Hybrids between *E. cardaminoides* and two diploid crop brassicas, *B. rapa* (AA) and *B. nigra* (BB), have shown intergenomic affinity, indicating a possibility of introgression of genes (Chandra *et al*. 2004). The present investigation is the first report on the production of an intergeneric hybrid between *E. cardaminoides* (Webb) OE. Schulz ($E^{cd}E^{cd}$, $2n = 18$, female parent) and *B. oleracea* var. alboglabra (CC, $2n = 18$, pollen parent). The study also analyses the extent of possible intergenomic affinity between E^{cd} and C genomes.

MATERIALS AND METHODS

The seeds of *E. cardaminoides* and *B. oleracea* var. alboglabra were obtained from the Indian Agricultural Research Institute, New Delhi, and were grown in a botanical garden. The wild species, *E. cardaminoides* is an annual herb which grows up to a height of 60 cm. Stem and leaves are pubescent. Leaves are lyrate and pinnatifid with acute apex. The plants flower in 35–40 days and remain in flowering for 75–80 days. Flowers are bright yellow and fruits are long and shattering. The cultivar *B. oleracea* var. alboglabra grows up to a height of 110 cm. Leaves are bluish green and glabrous. Plants flower in about 100 days and remain in flowering for almost 60 days. Petals are white and anthers have mauve tips. Fruits are long and do not shatter easily.

For pollination studies and hybrid embryo rescue, 208 flower buds of the female parent were emasculated a day before anthesis and bagged. The next day they were pollinated using pollen of the male parent and were subsequently re-bagged. Ten of the 208 pollinated pistils were excised 24 h after pollination and used for pollen germination and pollen tube growth studies by the aniline blue fluorescence technique (Shivanna & Rangaswamy 1992). The rest of the pollinated pistils (50 pistils in field and 148 for embryo-rescue) were left on the plant until drying or fruit maturity or used for embryo rescue methods (i.e. ovary, ovule or sequential culture of ovary and ovule). For embryo rescue methods, MS medium (Murashige & Skoog 1962) supplemented with casein hydrolysate (500 mg/l) was used. For ovary and sequential culture, 100 ovaries were excised 4–6 days after pollination (DAP), surface sterilized and then cultured. The cultured ovaries were dissected after 10 days and enlarged ovules were recultured on fresh MS basal medium. For ovule culture, 48 ovaries were excised 15–18 DAP and surface sterilized; ovules were then dissected out under aseptic conditions and cultured on MS basal medium.

The F_1 hybrids were multiplied *in vitro* using shoot tips and single node segments on MS medium supplemented with 0.2 mg/l benzylaminopurine and subsequently rooted on MS medium with 0.2 mg/l naphthalene acetic acid. *In vitro* raised F_1 hybrids were then hardened and transferred to field conditions for morphological and cytological studies. For meiotic studies flower buds were fixed in Carnoy's fixative (ethanol:chloroform:acetic acid, 6:3:1) for 24 h and anthers were squashed in 1% acetocarmine.

RESULTS AND DISCUSSION

The aniline blue fluorescence technique (Shivanna & Rangaswamy 1992) was used to study pollen germination and pollen tube growth. The cross, *E. cardaminoides* (female) × *B. oleracea* var. alboglabra (male), showed moderate pollen germination and pollen tube entry into the ovary region (Figure 1). The reciprocal cross did not show any pollen germination indicating unilateral incompatibility. Similar cases were observed in other intergeneric pollination studies in *Brassiceae* (Shivanna 1996). Field pollinations (in *E. cardaminoides* × *B. oleracea*) did not yield any seeds, indicating a possibility of post-fertilization barriers. To circumvent this, embryo rescue techniques (ovary, ovule and sequential culture of ovary and ovule) were applied and 13 hybrid seedlings were obtained. The cross efficiency of ovule culture was highest (16%), whereas that of ovary culture and sequential culture was 5.8% and 8.5%, respectively.

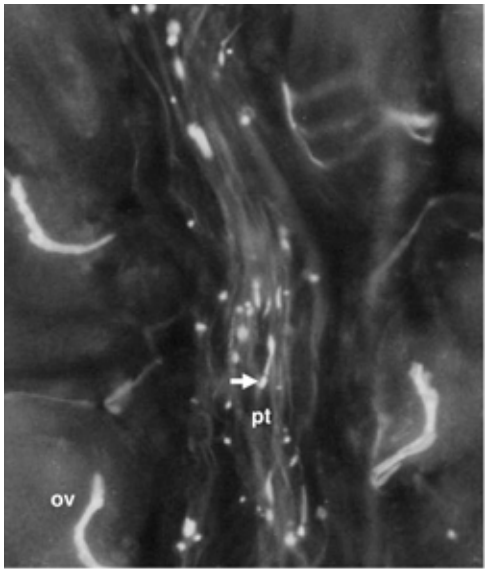

Figure 1. Pollination studies using the aniline blue fluorescence technique in *E. cardaminoides* × *B. oleracea* var. alboglabra; pollen tubes with callose (white arrow) have entered the ovary region

ov – ovule; pt – pollen tubes

The F_1 hybrid (Figure 2A) was morphologically intermediate between the parents in many of the characters (Table 1) except leaves and flowers (Figure 2B, C), which were more similar to *B. oleracea*. All plants of *E. cardaminoides* (12 plants) and F_1 hybrids (23 plants) growing in the field were healthy, without any symptoms of *Alternaria* blight, whereas 11/26 plants of *B. oleracea* var. alboglabra, growing in the vicinity, showed *Alternaria* blight infection. Anther dehiscence of F_1 hybrid was normal but produced only sterile pollen grains.

Cytology of pollen mother cells (PMCs) of the digenomic F_1 hybrid ($E^{cd}C$) revealed the expected number of chromosomes ($2n = 18$) at Metaphase I (Figure 3A, B) in all the 143 cells studied. There was a preponderance of univalents and a maximum of 3 bivalents were observed in the PMCs. One trivalent and one quadrivalent was also seen in 22% and 4% of the analysed cells, respectively. In haploids of *B. oleracea*, up to 3 bivalents were observed (Armstrong & Keller 1982). Considering the proposed basic chromosome number of the archetype of the tribe *Brassiceae* to be $x = 5$ or $x = 6$ (Quiros 1999), at least up to 2 bivalents due to autosyndesis are also expected in the haploid genome of *E. cardaminoides*. The number of bivalents in a digenomic hybrid may be exaggerated because, in addition to autosyndesis, preferential pairing between homoeologous chromosomes was also observed (Jenczewski

Table 1. Comparison of morphological characters of parents and the F_1 hybrid (*Erucastrum cardaminoides* × *B. oleracea* var. alboglabra)

Character	*E. cardaminoides*	F_1 hybrid	*B. oleracea* var. alboglabra
Plant height (cm)	53.8	90.8	108.6
Height of lowest branch (cm)	10.6	14.1	15.1
Basal leaf	green, petiolate, lyrate, pinnatifid, acute apex, pubescent, non glaucescent	bluish green, petiolate, lyrate, pinnatifid, obtuse apex, glabrous, non-glaucescent	bluish green, petiolate, lyrate with globose terminal lobe and 2–3 smaller lobes, obtuse apex, glabrous, glaucescent
Cauline leaf	subsessile, lanceolate, entire with smooth margin, obtuse apex, pubescent	subsessile, lanceolate, entire with wavy margin, obtuse apex, glabrous, non-glaucescent	sessile, oblong, entire with smooth margin, obtuse apex, glabrous, glaucescent
No. of days to flower	35–40	55–60	90–100
Duration of flowering	75–80	65–70	55–60
Petal size (mm)	9.0 × 4.0	13.2 × 6.0	15.0 × 8.0
Petal colour	yellow	white	white
Sepal size (mm)	5.0 × 1.0	8.0 × 3.0	9.0 × 5.0
Pistil length (mm)	6.0	11.1	19.1

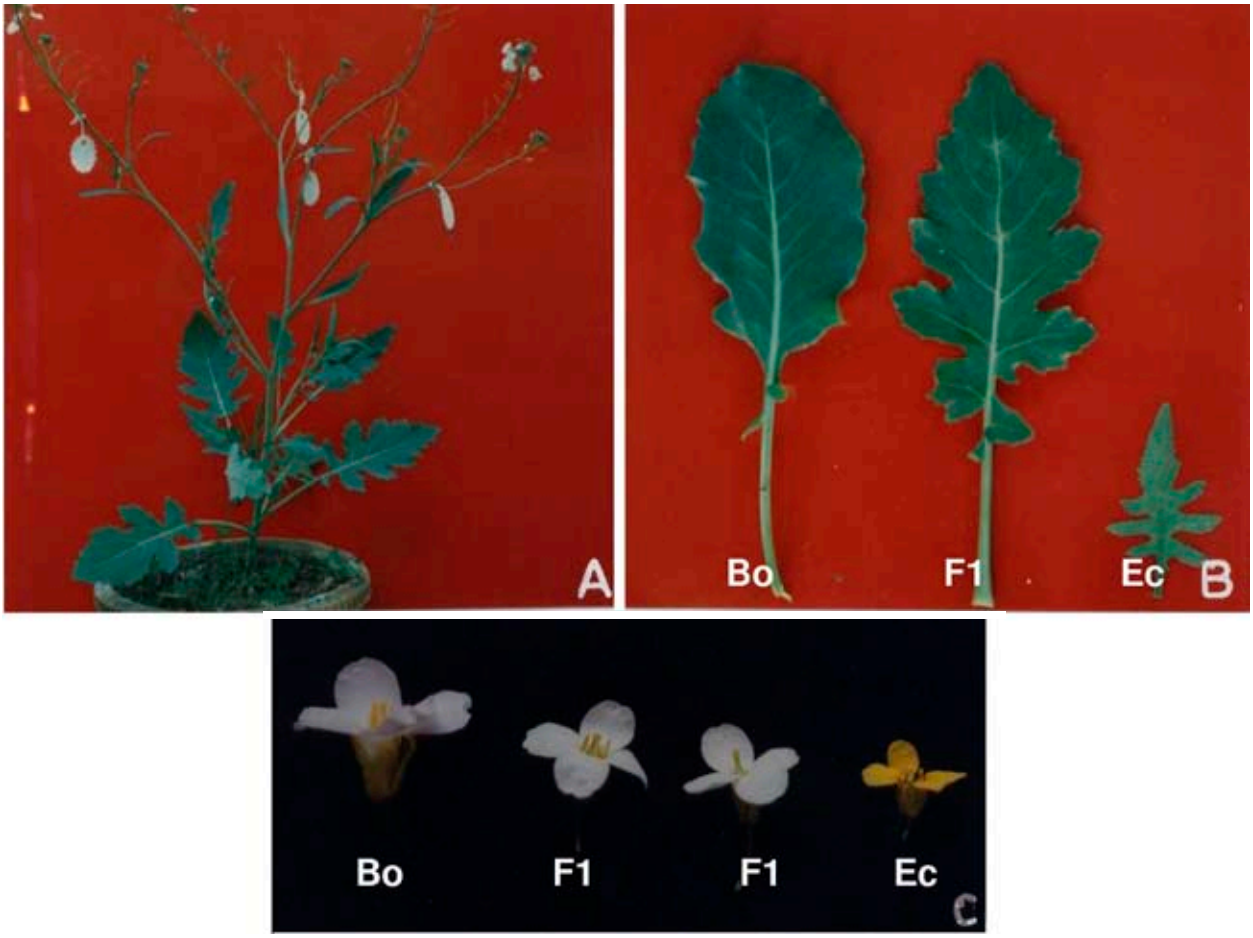

Figure 2. Morphology of F_1 hybrid plant (A); comparative leaf morphology (B) and floral morphology (C) of parents and F_1 hybrid

Bo – *B. oleracea* var. alboglabra, F_1 – F_1 hybrid, Ec – *E. cardaminoides*

et al. 2003; Chandra *et al*. 2004; Nicolas *et al*. 2008). However, the *E. cardaminoides* × *B. oleracea* hybrid showed lower than the expected number (at least 4 or 5) of bivalents. The F_1 hybrids *E. cardaminoides* × *B. rapa* ($E^{cd}A$) and *E. cardaminoides* × *B. nigra* ($E^{cd}B$) showed up to 5 bivalents and intergenomic affinity between E^{cd} and A/B genomes was indicated (Chandra *et al*. 2004). Limited homoeology between E^{cd} and C genomes is unexpected because considerable intergenomic homoeology was observed between E^{cd} and A/B genomes (Chandra *et al.* 2004). Moreover, since *Erucastrum* is phylogenetically closer to *B. rapa* (AA)*/B. oleracea* (CC) lineage than to *B. nigra* (BB) lineage (Lysak *et al*. 2005), higher homoeology is expected between E^{cd} and C genomes. It is possible that the presence of E^{cd} and C genomes together in the digenomic hybrid suppresses auto and/or allosyndetic bivalents. Although, the number of bivalents was low, the presence of a trivalent or a quadrivalent may indicate some homoeology between E^{cd} and C genomes. The production of amphidiploids of the digenomic hybrid can further clarify the intergenomic affinities between E^{cd} and C genomes. The F_1 hybrid was backcrossed to obtain BC1 progeny. The F_1 hybrid (as female parent) was also successfully used as bridge species to transfer the wild cytoplasm to *B. napus* and *B. carinata* (male parents).

The major implication of the present investigation is that the new intergeneric hybrid and the bridge cross hybrids have added to the existing genetic variability in crop brassicas. They can serve as new potential genetic resources which can be used to develop new CMS/ alloplasmic lines in crop brassicas.

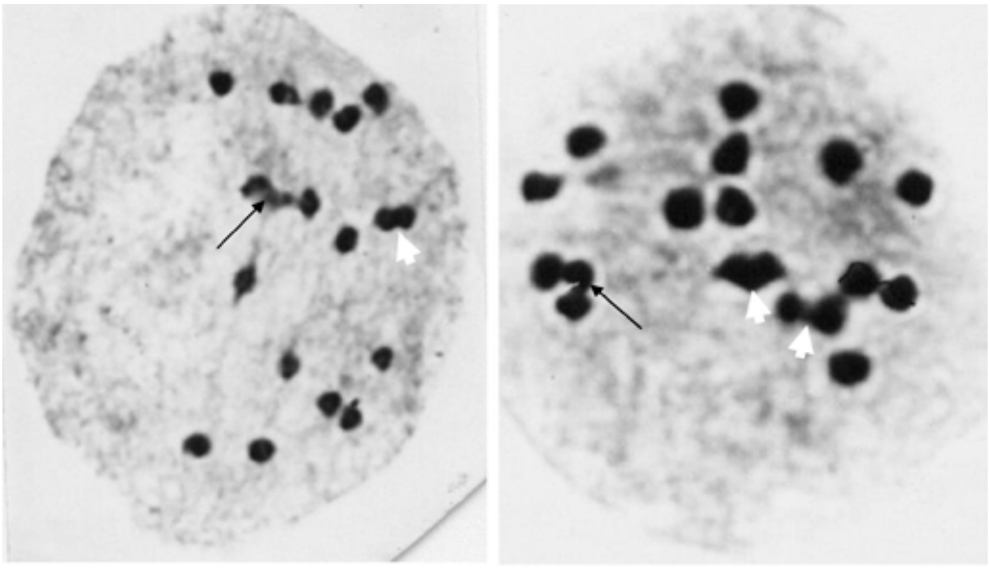

Figure 3. Meiosis in the F_1 hybrid, *E. cardaminoides* × *B. oleracea* var. alboglabra ($2n$ = 18); (A) 13I +1II +1III; (B) 11I +2II +1III; white arrows indicate bivalents, black arrows indicate trivalent

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References

Armstrong K.C., Keller W.A. (1982): Chromosome pairing in haploids of *Brassica oleracea*. Canadian Journal of Genetics and Cytology, **24**: 735–739.

Bang S.W., Sugihara K., Jeung B.H., Kaneko R., Satake E., Kaneko Y., Matsuzawa Y. (2007): Production and characterization of intergeneric hybrids between *Brassica oleracea* and a wild relative *Moricandia arvensis.* Plant Breeding, **126**: 101–103.

Bhat S.R., Kumar P., Prakash S. (2007): An improved cytoplasmic male sterile (*Diplotaxis berthautii*) *Brassica juncea*: identification of restorer and molecular characterization. Euphytica, **159**: 145–152.

Chandra A., Gupta M.L., Ahuja I., Kaur G., Banga S.S. (2004): Intergeneric hybridization between *Erucastrum cardaminoides* and two diploid crop Brassica species. Theoretical and Applied Genetics, **108**: 1620–1626.

Garg H., Banga S., Bansal P., Atri C., Banga S.S. (2007): Hybridizing *Brassica rapa* with wild crucifers *Diplotaxis erucoides* and *Brassica maurorum.* Euphytica, **156**: 417–424.

Gomez-Campo C. (1984): Studies on Cruciferae: XI *Erucastrum ifniense* Gomez-Campo, sp. nov., and its allies. Anales Jardin Botanico de Madrid, **41**: 83–85.

Gupta S.K., Sharma T.R., Chib H.S. (1995): Evaluation of wild allies of *Brassica* under natural conditions. Cruciferae Newsletter, **17**: 10–11.

Jenczewski E., Eber F., Grimaud A., Huet S., Lucas M.O., Monod H., Chevre A.M. (2003): Prbn, a major gene controlling homeologous pairing in oilseed rape (*Brassica napus*) haploids. Genetics, **164**: 645–653.

Lysak M.A., Koch M.A., Pecinka A., Schubert I. (2005): Chromosome triplication found across the tribe *Brassiceae*. Genome Research, **15**: 516–525.

Murashige T., Skoog F. (1962): A revised media for rapid growth and bioassays with tobacco tissue culture. Physiologia Plantarum, **15**: 473–497.

Nicolas S.D., Leflon M., Liu Z., Eber F., Chelysheva L., Coriton O., Chevre A.M., Jenczewski E. (2008): Chromosome 'speed dating' during meiosis of polyploid *Brassica* hybrids and haploids. Cytogenetic and Genome Research, **120**: 331–338.

Prakash S. (2001): Utilization of wild germplasm of *Brassica* allies in developing cytoplasmic male sterility-fertility restoration systems in Indian mustard *Brassica juncea.* In: Liu H., Fu T.D. (eds): Proc. Int. Symp. Rapeseed Science. Science Press, New York, 63–67.

Primard-Brisset C., Poupard J.P., Horvais R., Eber F., Pelletier G., Renard M., Delourme R. (2005): A

new recombined double low restorer line for the Ogu-INRA cms in rapeseed (*Brassica napus* L.). Theoretical and Applied Genetics, **111**: 736–746.

Quiros C.F. (1999): Genome structure and mapping. In: Gomez-Campo C (ed.). Biology of *Brassica coenospecies*. Elsevier Science, Amsterdam.

Rao G.U., Pradhan A.K., Shivanna K.R. (1998): Isolation of useful variants in alloplasmic crop brassicas in the cytoplasmic background of *Erucastrum gallicum*. Euphytica, **103**: 301–306.

Shivanna K.R. (1996): Incompatibility and wide hybridization. In: Chopra V.L., Prakash S. (eds): Oilseed and Vegetable Brassicas: Indian Perspective. Oxford & IBH Publishers, New Delhi, 77–102.

Shivanna K.R., Rangaswamy N.S. (1992): Pollen Biology: A Laboratory Manual. Springer-Verlag, Heidelberg.

Sigareva M.A., Earle E.D. (1997): Direct transfer of a cold-tolerant Ogura male-sterile cytoplasm into cabbage (*Brassica oleracea* ssp. *capitata*) via protoplast fusion. Theoretical and Applied Genetics, **94**: 213–220.

Warwick S.I., Francis A., L.A. Fleche J. (2000): Guide to Wild Germplasm of *Brassica* and Allied Crops (tribe *Brassiceae*, *Brassicaceae*). Agriculture and Agri-Food Canada, Ottawa.

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Corresponding author:

Dr. Aparajita Mohanty, University of Delhi, Department of Botany, Gargi College, Siri Fort Road, New Delhi-110049, India
tel.: + 91 11 26494544; fax: + 91 11 26494215, e-mail: aparajita.gargi@gmail.com