Alloplasmic line of *Brassica napus* L. with *Erucastrum canariense* cytoplasm is male sterile

S.S. Banga*, S.K. Banga, P.B. Bhaskar, I. Ahuja and B. Payal
Department of Plant Breeding
Punjab Agricultural University, Ludhiana – 141004, India
e-mail : surin11@rediffmail.com

ABSTRACT

To develop a new source of cytoplasmic male sterility for *Brassica napus*, an intergeneric amphiploid [*Erucastrum canariense* x *B. rapa* (2n=38; AAE^CE^C)] was used as female to initiate backcross substitution programme with *Brassica napus* (2n=38; AACC) cv. Ashai as the recurrent male parent. Male sterile and fertile plants were available in BC_1 and subsequent backcross generations. CMS plants had the expected euploid chromosome number and close morphological resemblance with the recurrent parent. No morphological aberrations were observed. The flowers of male sterile plants were small in size and had rudimentary anthers containing unstained pollen grains. There was no seed set on bagged selfed plants. Female fertility was normal in BC_3 plants as was evident from excellent seed set on crossing. All the *B. napus* accessions (29) evaluated were functional maintainer of the male sterility. Cytological analysis of fertile BC_1 segregants revealed variable chromosome number (2n=38 to 2n=46) with pollen stainability ranging from 15 to 49 percent. In BC_2, however, one nearly male fertile (93%) alloplasmic plant with chromosome number 2n=39 was recovered. It had the meiotic configuration of 2n=19 II + I or 2n=18 II + 1 III, indicating monosomic chromosome addition. Excellent pollen fertility of this plant was suggestive of the presence of *Rf* gene(s) on the extra chromosome. Occurrence of a trivalent with a mean frequency of 0.51 suggested the possibility of transferring *Rf* gene(s) from *E. canariense* chromosome to *B. napus* through homoeologous pairing with A/C genome chromosomes.

Key words : Cytoplasmic male sterility – backcross substitution – fertility restoration – cytoplasmic substitution – genomic affinity

INTRODUCTION

Many sources of cytoplasmic male sterility (CMS)-fertility restoration have been developed in *Brassica* oilseeds by exploiting enormous alloplasmic variability available in related species and genera (Prakash 2001). Male fertility of these alloplasmic CMS lines could be restored by the introgression of certain nuclear encoded genes, the fertility restorers, from the cytoplasm donor species. Notable example of the success of such a procedure had been the *ogura* CMS in *Brassica napus*, now widely being used to develop rapeseed hybrids. Inspite of the commercial success of current alloplasmic CMS systems (*ogura*, *tour*), efforts are being continued to develop new and functionally superior systems of pollination control. We now report the development of yet another alloplasmic CMS system for *Brassica napus* that is based on the cytoplasm of *Erucastrum canariense* (E^CE^C, 2n=18), a wild and weedy crucifer from Micronesian region in the Atlantic.

MATERIALS AND METHODS

The CMS (*can*) *B. napus* was developed by backcrossing an intergeneric amphiploid, *Erucastrum canariense* x *B. rapa* (2n=38; AAE^CE^C), as female with *B. napus* (AACC; 2n=38) cv. Ashai, as the recurrent male parent. In every backcross generation, plants phenotypically resembling *B. napus* were selected for next cycle of backcrossing. BC_1 male sterile and fertile plants were backcrossed again with the euplasmic recurrent parent. Crosses of the prospective CMS plants with a large number of *B. napus* germplasm lines were also attempted to identify the maintainers of male sterility expression. For cytological analysis squash preparation of anthers fixed in Carnoy's-II solution were studied. Pollen fertility was estimated through staining with 2% acetocarmine as well as that from seed set on bagged selfed plants.

RESULTS

Alloplasmic male sterile *B. napus* plants were similar to euplasmic recurrent parent in morphology and plant vigour. No adverse effect on agronomic performance of plants was apparent. Male sterility manifested in flowers with rudimentary anthers harboring small and unstained pollen grains. This was in contrast to fully developed anthers and deeply stained pollen grains of the euploid parent. Male sterility was complete and stable throughout the crop season. There was no seed set on bagged selfed plants. Controlled experiments did not reveal any effect of photoperiod and temperature on male sterility expression. Floral nectaries were almost normal in size. Female fertility was very low; as revealed by poor seed set on artificial pollination, in BC_1 plants. Dramatic improvement, in female fertility was evident with BC_2/BC_3 CMS plants showing excellent seed set on test crossing or open pollination. Cytological analysis of BC_3 CMS plants suggested a normal meiosis and expected chromosome number (2n=38). Complete male sterility maintenance was observed in 29 F_1 progenies resulting from crosses of CMS plants with euplasmic *B. napus* accessions. Male fertility in fertile segregants ranged from 15 to 49 percent in BC_1 generation. Cytological analysis of these male fertile plants indicated aberrant chromosome number ranging from 2n=38 to 2n=46. In the BC_2 generation, however, one nearly male fertile (93%) alloplasmic plant with the chromosome number 2n=39 could be recovered. It had the meiotic configuration of 2n=19II+1I or 2n=18II+11II, indicating monosomic chromosome addition. Mean trivalent frequency was 0.51.

DISCUSSION

Cytoplasmic male sterility in alloplasmic plants is considered to be an outcome of impaired nucleo-cytoplasmic harmony between the endogenous nucleus and alien cytoplasm. Wild crucifers have proved to be excellent sources of cytoplasmic male sterility in crop brassica species (Prakash 2001). *E. canariense* has been previously demonstrated to possess substantial intergenomic affinity with 'A' genome of *B. rapa* (Bhaskar *et al*. 2002) as well as the 'C' genome of *B. oleracea* (Harberd and McArthur 1980); the nuclear genome donors for the amphiploid *B. napus* (AACC). Successful induction of CMS, however, shows cytoplasmic divergence between the *B. napus* and *E. canariense*. As was expected, the euplasmic *B. napus* germplasm maintained male sterility since recessive plasmon sensitive alleles are known to occur with high frequency in populations having normal plasmotype. Excellent pollen fertility in the plant carrying monosomic chromosome addition was suggestive of presence of *Rf* gene(s) on the extra chromosome. The extra chromosome in this monosomic addition line occurred as a trivalent with a frequency of 0.51. This emphasized the possibility of transferring *Rf* gene(s) from *E. canariense* chromosome to *B. napus* through homoeologous pairing with A/C genome chromosome.

Summarizing, the *canariense* (*can*) CMS appears to have excellent commercial prospects due to stable male sterility expression, absence of sterilizing cytoplasm related morphological abnormalities, abundant male sterility maintainers and distinct probability of developing male sterility restorers.

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