DEVELOPMENT OF IMPROVED CYTOPLASMIC MALE STERILE LINES IN *BRASSICA* THROUGH SOMATIC CELL HYBRIDIZATION

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ABSTRACT

'Tour' and 'oxy' are two stable alloplasmic CMS systems in *B. juncea*. In addition to male sterility these CMS systems are associated with other morphological and floral abnormalities. In order to rectify these problems, somatic cell hybridization was carried out to substitute the alien chloroplast and to generate mitochondrial recombinants. The protoplasts of CMS *B. juncea*, AABB (for both 'tour' and 'oxy') were fused with normal *B. oleracea*, CC to synthesize AABBCC somatic hybrids. A large number of somatic hybrids were raised and transferred to the field. These hybrids segregated for fertility and sterility and also show variations in floral morphology. The phenotypic and molecular characterization of these hybrids has been carried out and their possible significance has been discussed.

INTRODUCTION

Cytoplasmic male sterility (CMS) is a maternally inherited trait and is encoded by the mitochondrial DNA (mtDNA). It occurs either spontaneously in a normal population or can be induced by the transfer of the cytoplasm from a wild or related species into the nuclear background of a cultivated species by repeated backcrossing. The latter one is known as alloplasmic CMS. In majority of the crop plants, the alloplasmic CMS has not been used in practice because of depressive effects of alien cytoplasm on plant and flower morphology. It poses frequent problems in developing an ideal CMS line and identification of the restorer(s) from existing germplasm of cultivated species.

In *Brassica* different sources of alloplasmic CMS have been reported. Two important sources among them are 'tour' (Pradhan *et al.*, 1991) and 'oxy' (Prakash and Chopra, 1990) which have been developed by transferring the cytoplasm of *B. tournefortii* and *B. oxyrrhina* respectively into nuclear background of different cultivated *Brassica* species. In addition to male sterility, these CMS are associated with other abnormalities like chlorosis (due to chloroplast-nuclear incompatibility), petaloid anthers, reduction in the size of flowers and buds, petalless flowers (in some nuclear background of *B. juncea*) and reduction in the main shoots and pod length. These could be attributed to incompatibility at many *loci* between alien cytoplasm and the nuclear genome of cultivated species. Replacement of alien chloroplast with cultivar chloroplast and replacement of most of the incompatible segments of the alien mtDNA with cultivar mtDNA excepting the *locus* (or

loci) conferring the CMS character may provide a solution to this problem. Since protoplast fusion technique provides an unique opportunity to combine the organelles of two parents and allows segregation (chloroplast) and recombination (mitochondria) of organelles in somatic hybrids, it could be possible to obtain novel combinations of chloroplast and mitochondria as mentioned above. Using this technique, abnormalities such as chlorosis and poor nectary development observed in 'Ogura' CMS in *B. napus* have been rectified (Pelletier *et al*, 1983).

In order to develop ideal male sterile lines by replacement of chloroplast and generating mitochondrial recombinants we have synthesized somatic hybrids with genomic configuration of AABBCC by fusing the protoplasts of CMS *B. juncea*, AABB (for both 'tour' and 'oxy') with protoplasts of normal *B. oleracea*, CC. The AABBCC hybrids with 'tour' CMS were analysed for nuclear and organelle genome composition and were backcrossed to three digenomic *brassica* species *B. juncea, B. napus* and *B. carinata* for transfer of cytoplasmic variability.

EXPERIMENTAL

Materials and methods

Shoot protoplasts of hygromycin resistant 'oxy' and 'tour' CMS *B. juncea*, AABB *hpt*$^+$ were fused with protoplasts of phosphinotricin resistant normal *B. oleracea*, CC *bar*$^+$. The selectable marker lines used in this study enabled a very efficient selection of hybrid colonies and plants. Protocols for protoplast fusion and molecular characterization have been described in Mukhopadhyay *et al.* (1994). Table 1 shows the details of the synthesis of two different AABBCC somatic hybrids and their stages of development.

TABLE 1. Different AABBCC somatic hybrids synthesized

Fusion partners		Composition of somatic hybrids	No. of hybrid colonies	No. of independent regenerants
Parent A	Parent B			
AABB *hpt+* *tour* CMS	CC *bar+*	AABBCC	976 (59)	82*
AABB *hpt+* *oxy* CMS	CC *bar+*	AABBCC	1381(19)	29**

Figure in the parenthesis indicates the number of regenerating colonies.

* 78 out of 82 regenerants have been backcrossed to three different digenomic *Brassica* species.

** 29 AABBCC with 'oxy' CMS have been transferred to the net house during the current growing season and remaining colonies are kept for regeneration *in vitro*.

AABBCC hybrids with 'tour' CMS

Majority of the hybrids were vigorous in growth and flowered within three months after transfer to the net house. The overall morphology was intermediate between the two parents. On the basis of floral morphology they were broadly classified into 4 group, (i)

male sterile with normal flower, (ii) male sterile with abnormal flower (petaloid, petalless, curved stigma, etc.), (iii) male fertile with normal flower and (iv) male fertile with abnormal flower (petalless, curved and thick stigma, etc.). Most of them set seeds after backcrossing to different digenomic *Brassica* species. Backcross progeny have been sown in the net house during the current growing season.

We analysed 78 independent hybrids for chloroplast (cp) and mitochondrial (mt) composition and it was observed that 46 out of 78 had rearranged/recombined mtDNA of varying degree but none had rearranged cpDNA. 24 hybrids showed replacement of *B. tournefortii* chloroplast and interestingly all these 24 hybrids had rearranged/recombined mtDNA with a predominance of *B. tournefortii* specific bands. 28 hybrids had cp- and mt-DNA of *B. oleracea* out of which 12 exhibited recombination in mt-DNA. This indicates that enough desirable variability in the organelle genome could be created by protoplast fusion.

The lead was from sunflower (*Helianthus annuus*) CMS which is a alloplasmic CMS system having the cytoplasm of *H. petiolaris*. In this CMS system, there are large number of fertility restorer lines available in sunflower resulting in commercial production of hybrid seed and the CMS lines apparently do not exhibit any depressing alloplasmic effects. Organelle genome analysis of CMS and normal lines indicated that cp DNA of both the lines are similar and mt DNA differs for a 17 kb region (one inversion and one deletion or duplication) containing orf 522 which could be responsible for CMS phenotype (Monegar *et al.*, 1994). In the present investigation we plan to simulate a similar situation that has been observed in CMS system of sunflower. The advantages of synthesizing AABBCC hybrids are (1) they can be maintained sexually and (2) can be crossed to all the cultivated digenomic *Brassica* species so that the organelle genome variability that are created by protoplast fusion can be transferred simultaneously to all the three species. The improved male sterile lines thus obtained could be subsequently tested against existing germplasm of the cultivated species to identify the restorer gene(s).

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