Theor Appl Genet (2006) 114:93–99
DOI 10.1007/s00122-006-0413-0

ORIGINAL PAPER

A new cytoplasmic male sterility system for hybrid seed production in Indian oilseed mustard *Brassica juncea*

Y. S. Sodhi · A. Chandra · J. K. Verma · N. Arumugam · A. Mukhopadhyay · V. Gupta · D. Pental · A. K. Pradhan

Received: 1 October 2005 / Accepted: 8 September 2006 / Published online: 12 October 2006

Abstract We report a novel cytoplasmic male sterility (CMS) system in *Brassica juncea* (oilseed mustard) which could be used for production of hybrid seed in the crop. A male sterile plant identified in a microspore derived doubled haploid population of re-synthesized *B. napus* line ISN 706 was found to be a CMS as the trait was inherited from the female parent. This CMS, designated '126-1', was subsequently transferred to ten different *B. juncea* varieties and lines through inter-specific crosses followed by recurrent backcrossing. The F_1s of inter-specific crosses were invariably partially fertile, but irrespective of the variety/line used, the recipient lines became progressively male sterile over five to seven generations and could be maintained by crossing the male sterile lines with their normal counterparts. The male sterile lines were found to be stable for the trait under both long and short day conditions. CMS lines when crossed with lines other than the respective maintainer line were restored for fertility, implying that any variety could act as a restorer for '126-1' cytoplasm in *B. juncea*. These unique features in maintenance and restoration of CMS lines coupled with near normal floral morphology of the CMS lines have allowed the use of '126-1' cytoplasm for hybrid seed production. The uniqueness of '126-1' has been further established by Southern hybridization with mitochondrial DNA probes and by a histological study of the development of male sterile anthers.

Communicated by H. C. Becker.

Y. S. Sodhi (✉) · A. Chandra · J. K. Verma · N. Arumugam · A. Mukhopadhyay · V. Gupta · D. Pental · A. K. Pradhan
Centre for Genetic Manipulation of Crop Plants,
University of Delhi South Campus,
Benito Juarez Road, 110021 New Delhi, India
e-mail: sodhiys@hotmail.com

D. Pental · A. K. Pradhan
Department of Genetics,
University of Delhi South Campus,
Benito Juarez Road, 110021 New Delhi, India

Introduction

Heterosis breeding could be used for enhancing crop productivity in *Brassica juncea*, a major oilseed crop of the Indian subcontinent. Divergent gene pools and their possible potential for heterosis breeding has already been shown in *B. juncea* (Pradhan et al. 1993; Srivastava et al. 2000). However, besides the identification of suitable combiners, a pollination control mechanism for the production of hybrid seed on a large scale is also required. Cytoplasmic male sterility (CMS), a phenotypic manifestation of incompatibility between nuclear and cytoplasmic genomes, is a maternally inherited trait that has been successfully exploited as an effective pollination control mechanism for the production of hybrid seed in many crop plants (reviewed by Havey 2004).

A number of CMS systems of spontaneous and alloplasmic origin have been reported in *Brassica* species (Budar et al. 2004). Spontaneous CMS arises without any intentional intervention, an example being 'Polima' CMS in *B. napus* (Fu 1981). Recently, Liu et al. (2005) reported a spontaneous CMS in a mutant line of a *B. napus* cultivar Xiangyu 13. Several alloplasmic CMS-systems also have been reported in *B. juncea*. Alloplasmic lines of *B. juncea* containing cytoplasm of *B. oxyrrhina* (Prakash and Chopra 1990), *B. tournefortii* (Pradhan et al. 1991; Arumugam et al. 1996), *Diplotaxis siifolia* (Rao et al. 1994), *Trachystoma ballii* (Kirti et al. 1995a),

Raphanus sativus (Kirti et al. 1995b), *Moricandia arvensis* (Prakash et al. 1998) and *Erucastrum canariense* (Prakash et al. 2001) have been found to be male sterile. However, it has not been possible to exploit these alloplasmic CMS systems effectively for heterosis breeding, either due to chlorosis and floral abnormalities in the CMS lines or due to lack of lines within *B. juncea* which could restore male fertility of the CMS lines. For many of the alloplasmic CMS systems, which were corrected for floral abnormalities and chlorosis by somatic hybridization (Kirti et al. 1995b; Arumugam et al. 1996; Arumugam et al. 2000), attempts have also been made to introgress the restorer gene(s) from the alien species which have been the cytoplasm-donors. Most of the attempts to transfer restorer functions from the cytoplasm donor species have been beset with the problem of linkage drag. Kirti et al. (1997) reported a restorer plant for *T. ballii* CMS in *B. juncea* with 90% pollen viability. However, the identified restorer plant showed leaf serration of *T. ballii*, intermediate flower morphology, contorted pods and yellow cylindrical seeds typical of *T. ballii*, indicating an imperfect transfer of the restorer gene from the alien species. Proper restoration could not be achieved for the alloplasmic CMS lines of *B. juncea* containing *E. canariense* cytoplasm (Prakash et al. 2001). Restored plants showed 90% pollen viability but suffered from a reduction in female fertility. Prakash et al. (1998) reported transfer of restoration function with 96% pollen viability for a CMS system derived from *M. arvensis*. However, the restorer plant exhibited severe chlorosis similar to that observed in the CMS plants and also had reduced female fertility. Thus, none of the alloplasmic CMS systems tested in *B. juncea* have proved to be adequate for hybrid seed production.

In this paper, we report the identification of a novel CMS system (named '126-1') in *B. napus* and its transfer to *B. juncea* which could be used for hybrid seed production in this crop. CMS '126-1' was initially identified as a spontaneous CMS in a microspore derived doubled haploid population of *B. napus* and was subsequently transferred to *B. juncea* lines through inter-specific crosses. We also report in this paper the maintenance and restoration characteristics of *B. juncea* lines containing '126-1' cytoplasm. The CMS in *B. juncea* has also been characterized through Southern hybridization and developmental histology of the male sterile anthers.

Materials and methods

The experimental lines and varieties of *B. juncea* used in this study are BNF-5, D-205, D-247, DYJ-III, EH-2 (breeding lines) and Pusa bold, Varuna, Pusa agrani, TM-4, TM-18 (varieties). The synthetic *B. napus* line ISN 706 used in this study was derived from a cross between *B. campestris* ssp. *oleifera* var. brown sarson and *B. oleracea* var. *botrytis* cv. 'Pusa Katki' (Prakash and Raut 1983). The germplasm used for the study was grown under short-day conditions from October to March in Delhi (normal growing season) and under long-day conditions from May to September at Leh for off-season generation advance. For microspore culture, *B. napus* ISN 706 plants were grown under controlled environment conditions in a growth chamber (relative humidity 80%); initially under 10 h/14 h and 20/15°C day/night for 4–5 weeks till the onset of flowering, followed by 14 h/10 h, 10/5°C day/night for a minimum of 15 days prior to the isolation of microspores. Production of doubled haploids (DH) from microspores of *B. napus* ISN 706 was carried out following Mollers et al. (1994). The ploidy status of putative DH plants was analyzed following Arumuganathan and Earle (1991) using a ploidy analyzer. Confirmed DH plants were grown in the field during the normal growing season. The male sterility status of the putative CMS plants was studied by monitoring the seed set in bagged inflorescences.

To study the development of anthers and microspores, entire inflorescence containing up to 1–2 open flowers from both male sterile and normal plants were fixed either in 10% ethanol or in Karnovsky's fluid following O'Brien and McCully (1981). Buds ranging in size from 1 to 7 mm were used for squash preparation to study microspore development as well as for histological preparations to study the overall development of the anther. For squash preparations, anthers from buds at selected stages were dissected, crushed between slide and coverslip in a drop of Alexander's stain (Alexander 1969), sealed with nail-enamel and incubated overnight at 30°C. Squash preparations were stained with 0.1% acetocarmine to study meiosis in the CMS and normal lines. Pollen viability tests were conducted by staining microspores with fluorescein di-acetate (FDA) following the protocol of Heslop-Harrison et al. (1984). For histological studies, buds fixed in Karnovsky's fluid were dehydrated through ethanol series, embedded in glycol methacrylate which was polymerized at 60°C and the block was cut into 3–4 μm semithin sections. Slides were stained in Toluidine blue O (O'Brien and McCully 1981). Autoflourescence of sporopollenin and lignified tissue was visualized by UV transmission microscopy.

For the RFLP profiling of '126-1' CMS, total DNA was isolated from the experimental lines using the CTAB method described by Rogers and Bendich

(1994). DNA was digested with *Eco*RI restriction endonuclease in accordance with the recommendations of the manufacturer, electrophoresed on 0.8% agarose (Sigma) gels and transferred to nylon membranes (Amersham, USA). Southern hybridizations were carried out using three different mitochondrial DNA cosmid clones ($pCos^{13}$, $pCos^{17}$ and $pCos^{42}$) containing *B. oxyrrhina* mitochondrial DNA as inserts (Arumugam et al. 1996).

Results

Origin of '126-1' CMS in *B. napus*

In a population of about 5,000 doubled haploid plants of *B. napus* ISN 706, one plant was found to be male sterile. This individual plant was normal in its phenotype in all aspects except that anthers were reduced in size and did not contain viable pollen. This male sterile plant was crossed with normal ISN 706 and the trait of male sterility was inherited by all the progeny plants showing thereby that the trait was inherited from the female parent. This CMS system was designated '126-1' and has been maintained stably in *B. napus* ISN 706 for more than seven backcross generations.

Transfer of '126-1' CMS to *B. juncea*

B. napus ISN 706 with '126-1' cytoplasm was crossed with different *B. juncea* lines namely BNF-5, D-205, D-247, DYJ-III and EH-2 and some popular Indian varieties namely Pusa bold, Pusa agrani, Varuna, TM-4 and TM-18. The F_1s of the inter-specific crosses were observed to be partially fertile. Successive backcrosses to the respective recurrent parent led to progressive decrease in the percentage of viable pollen grains eventually leading to complete male sterility. The number of backcross generations required for the expression of complete male sterility varied for the different lines. Lines BNF-5, D-205, D-247, Pusa agrani, TM-4 and TM-18 became completely male sterile by the BC_4 generation; EH-2 and Pusa bold were sterile by BC_5 and Varuna required seven backcrosses to achieve complete male sterility. No seed set was observed on selfing in the male sterile lines and the pollen of such lines did not show any fluorescence when stained with FDA. The male sterile line of Pusa bold with '126-1' cytoplasm has been grown for six generations both in Delhi under short day and at Leh under long day conditions and found to be stable for the trait of male sterility. Flowers of the male sterile line showed normal floral morphology. However, the anthers were lighter in color and devoid of viable pollen grains. Seed set on male sterile plants was comparable with normal counterpart indicating no female sterility.

In order to search for a restorer of '126-1' CMS in *B. juncea* germplasm, crosses were made between the CMS and normal *B. juncea* lines. It was observed that crosses between any one of the '126-1' CMS lines and any normal line, except the respective maintainer line, produced fully fertile F_1 plants with a pollen viability of more than 90%. Such hybrids set normal seed on selfing. Thus, it was observed that any variety could act as a restorer as well as a maintainer for the cytoplasm '126-1'.

Microspore development and histology of anthers

Meiosis was studied in anthers of normal and male sterile flowers by making squashes and staining with acetocarmine. Squash preparations of anthers taken from the flower buds (1–3 mm) of the normal and CMS lines showed normal meiosis leading to normal development of microspores till the tetrad stage. Post-tetrad stages were studied by staining of the squash preparations with Alexander's stain. Post-tetrad stage microspores in normal flower buds (4–7 mm) showed progressive development of healthy pollen grains with dense cytoplasm that stained deep red and well developed tricolpate exine that stained green. In contrast, microspores from flower buds of male sterile plants at comparable stages of development, showed progressive degeneration through necrosis of cytoplasm and collapse of exine.

The developmental histology of normal and male sterile anthers was studied to ascertain the stage at which degeneration of microspores and other anther tissue occurred. Sections of the anthers of normal buds collected just prior to anthesis showed well developed, intact tapetum layer surrounding fully formed microspores in the locules (Fig. 1a). In comparison, anthers of the CMS line showed plasmolysed microspores with irregular exine. Cells of the tapetum layer were intact at this stage but showed some abnormalities. The cytoplasm stained more heavily, there was vacuolation in the cells and intercellular spaces were more prominent (Fig. 1b). It can be concluded that the degeneration of microspores preceded the degeneration of the tapetal layer.

Degenerating tapetum in *Brassicaceae* is characterized by a high lipid content, which can be visualized by its autofluorescence under UV light. Cross sections of normal anthers at post-anthesis stages showed that the lipid material from the degenerating tapetum was deposited around the exines of fully developed microspores (Fig. 1c). In comparison, sections of male

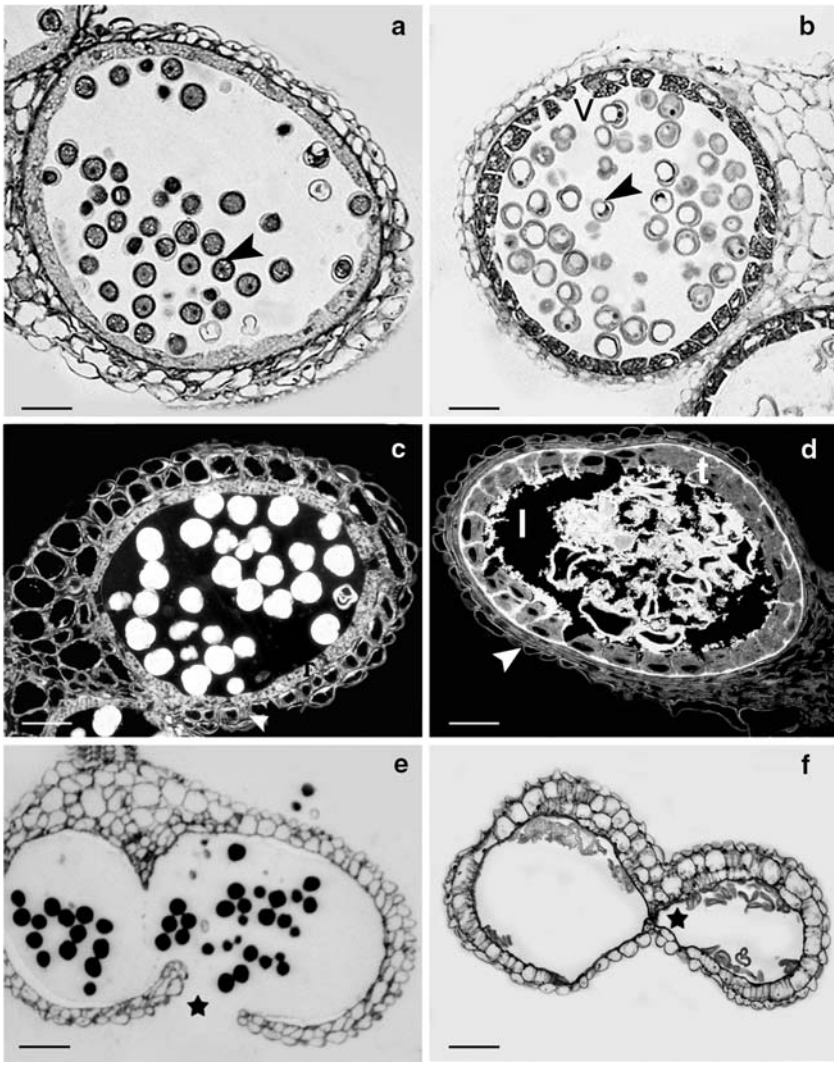

Fig. 1 Light micrographs of thin (3–4 μm) transverse sections of floral buds from normal plants of *B. juncea* var. Pusa bold (**a**, **c**, **e**) and male sterile plants of *B. juncea* Pusa bold with '126-1' cytoplasm (**b**, **d**, **f**). **a** Anther from a male fertile flower showing well developed microspores (*arrow head*) surrounded by a well differentiated tapetum and exine. **b** Anther from a male sterile flower showing degenerated microspores with plasmolysed cytoplasm (*arrow head*), irregular exines and vacuolated tapetal cells (*V*). **c** A normal anther showing bright auto-fluorescence of lipids in the microspores and endothecial layer showing well differentiated secondary wall thickenings (*arrow head*). **d** An anther from a sterile bud showing lipidic material (*l*) leaking from the tapetal (*t*) cells and degenerated microspores. **e** An anther of a normal plant at the dehiscence stage showing properly developed microspores and a well defined stomium (*asterisk*). **f** Indehiscent anther of a male sterile flower showing absence of the stomium (*asterisk*) and collapsed microsporangia containing empty sporoderms (magnification for **a**, **b**, **c**, **d**, bar = 1 cm = 58.3 μm, for **e**, **f** bar = 1 cm = 87.5 μm)

sterile anthers at similar stages of development showed deposition of the lipid material into the outer periphery of the tapetal layer (Fig. 1d) indicating impairment in intercellular trafficking of the lipid materials in the male-sterile anthers. At this stage the anther locules of the male-sterile contained only empty exines and microspores had completely degenerated.

Cross sections of normal anthers before anthesis also showed well developed endothecial layers with secondary wall thickenings (Fig. 1c). At maturity, the dehisced anthers were characterized by the presence of stomium and an absence of interlocular septum (Fig. 1e). In the male sterile anthers the endothecial layer was made up of only flattened cells which lacked secondary wall thickenings (Fig. 1d). At maturity, male-sterile anthers showed an absence of stomium and a functional endothecial layer (Fig. 1f). The interlocular septum did not rupture and the anthers remained indehiscent.

RFLP profiling of '126-1' CMS

Southern hybridization of DNA extracted from *B. napus* ISN 706, ISN 706 with '126-1' cytoplasm and different *B. juncea* normal and CMS '126-1' lines was carried out with three cosmid clones ($pCos^{13}$, $pCos^{17}$ and $pCos^{42}$) containing mitochondrial DNA inserts of *B. oxyrrhina*. The hybridization patterns of CMS lines of *B. napus* and *B. juncea* were similar and could be distinguished from the normal lines on the basis of the RFLP pattern generated by Southern hybridization. The RFLP pattern of mitochondrial DNA hybridized to $pCos^{13}$ has been shown in Fig. 2a. Examination of the RFLP patterns of mitochondrial DNA of different CMS systems ('Ogura', 'Oxy', 'Diplotaxis', 'Tour' and 'Moricandia') and CMS '126-1' in Pusa Bold background with $pCos^{13}$ mitochondrial DNA cosmid clone revealed that the RFLP pattern of '126-1' CMS was distinct from all the other tested CMS systems. This distinct RFLP pattern could be readily used for the identification of '126-1' CMS (Fig. 2b).

Discussion

Most of the CMS systems that have been described in *Brassica* species are alloplasmic in nature. Of these only 'Ogu' CMS in *B.* napus has been used for hybrid seed production with some success. 'Ogu' CMS was first discovered in *R. sativus* as a spontaneous mutant (Ogura 1968). This CMS was subsequently transferred to *B. napus* by wide hybridization and recurrent backcrossing. Resultant lines were chlorotic and suffered from floral abnormalities and bud death (Bannerrot et al. 1974). Problems of chlorosis and floral abnormalities were corrected by somatic hybridizaion (Pelletier et al. 1983). As restorer genes could not be identified in *Brassica* species, a restorer locus identified in *R. sativus* was transferred from radish to *B. napus* by hybridization followed by recurrent backcrossing to *B. napus* lines (Heyn 1976). Resultant restorer lines were agronomically inferior as these contained undesirable *Raphanus* characters due to linkage drag (Delourme and Renard 1988). Further improvement came from

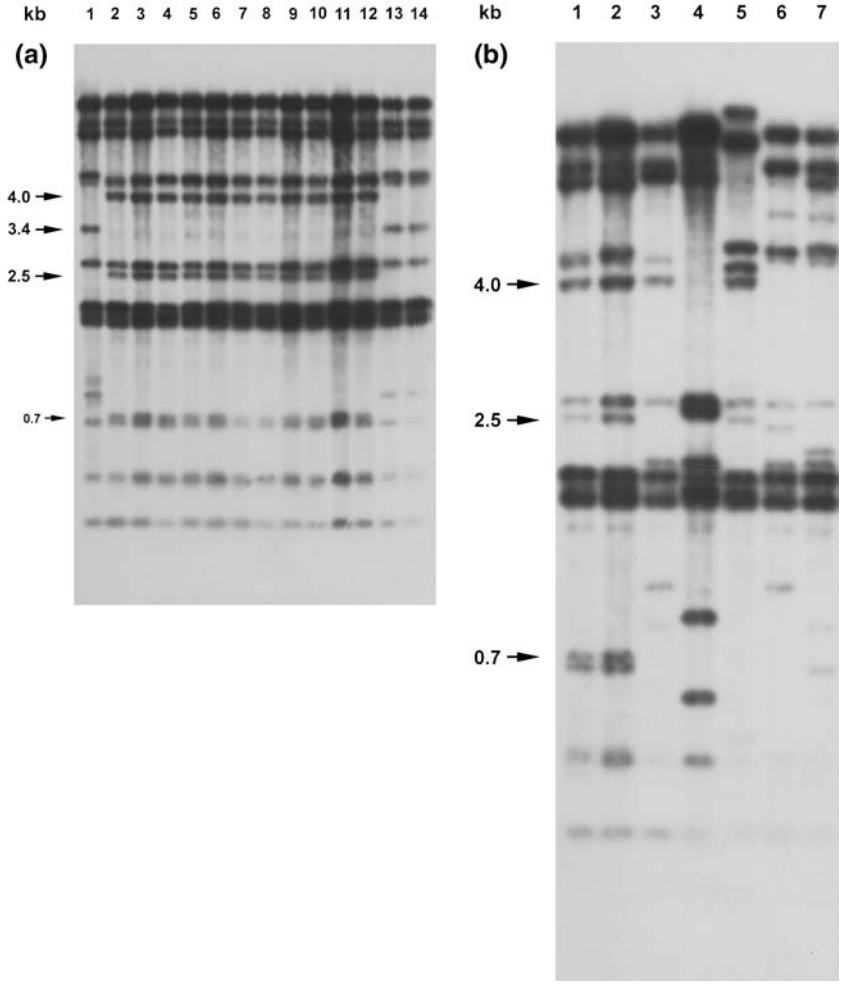

Fig. 2 RFLP profiling of '126-1' CMS and some of the other CMS systems described in *B. juncea*. Total DNA was digested with *Eco*RI and hybridized to cosmid clone $pCos^{13}$ containing mitochondrial DNA inserts from *B. oxyrrhina*. **a**: *Lane 1* normal *B. napus* ISN 706, *lane 2* '126-1' *B. napus* ISN 706; *lanes 3–12* '126-1' CMS lines of *B. juncea* BNF-5, D-205, D-247, DYJ-III, Pusa agrani, TM-4, TM-18, Varuna, Pusa bold and EH-2, respectively; *lanes 13, 14* normal *B. juncea* lines EH-2 and Pusa bold, respectively. All the CMS lines showed 4.0 kb, 2.5 kb and 0.7 kb bands specific to '126-1' CMS. **b** *Lane 1* '126-1' CMS *B. napus* ISN 706, *lanes 2–7* '126-1' CMS, Ogura CMS, Oxy CMS, Diplotaxis CMS, Tour CMS and Moricandia CMS in the nuclear background of *B. juncea* cv. Pusa bold, respectively

selecting rare recombinants with reduced linkage drag (Delourme et al. 1991). 'Ogu' CMS has been transferred to *B. juncea* through somatic hybridization to correct the chlorosis problem (Kirti et al. 1995b) but there is no report of transfer of the restorer gene(s). A number of other CMS systems described in *B. juncea* have been found inadequate for hybrid seed production due to lack of proper restorer lines.

The '126-1' CMS system described in this study is of a special nature, both in terms of maintenance and restoration. This CMS has a distinct RFLP profile and a distinct developmental pattern. The degeneration of microspores in '126-1' CMS occurs at the late anther development stage and floral morphology is near normal except for the presence of shorter indehiscent anthers that contain aborted pollen.

The development of anthers in plants with '126-1' CMS differs significantly from the development of anthers described for 'Ogu' CMS in *B. napus* (Gourret et al. 1992). In '126-1' CMS, normal development of microspores continues till the tetrad stage. The degeneration of microspores in '126-1' CMS seems to be independent of the degeneration of the tapetum at least in the initial stages. In comparison, the degeneration of the tapetum seems to precede microspore abortion in the 'Ogu' cytoplasm. The leakage of lipid materials into the outer periphery of the tapetal layer is also a characteristic feature of '126-1' CMS. However, '126-1' CMS and 'Ogu' CMS show similarities in terms of the indehiscent nature of the anther at maturity. As anatomical details of other CMS systems described in *Brassica* species is not available, a comparison cannot be made with other systems.

There are many advantages in the utilization of '126-1' CMS for hybrid seed production. In field studies we have observed extensive seed set on the male sterile lines in maintainer plots and hybrid seed production plots (approximately 95% of the normal lines). The most unique and agronomically interesting feature of '126-1' CMS in *B. juncea* is that any line can act as a fertility restorer in the F_1 generation and can also be used as a maintainer after a few generations of backcrossing. The molecular mechanism underlying CMS '126-1' could be unique and is currently being studied.

The first *B. juncea* hybrid (DMH-1) developed with '126-1' cytoplasm has given around 30% heterosis over the best national and regional checks in multi-site trials conducted in the north-western states of India where mustard is grown extensively during the winter growing season. These trials were conducted for two consecutive seasons in small plot replicated trials and in the 2004–2005 growing season in farmer's fields (data not presented). As the male sterile lines with '126-1' cytoplasm are stable both under long day and short day conditions, CMS '126-1' besides its use in India may also be of value in developing *B. juncea* hybrids for regions where *B. juncea* is grown in the summer season.

Acknowledgments This work was supported financially by the Dhara Vegetable Oil & Food Company Ltd. (DOFCO), a subsidiary of the National Dairy Development Board (NDDB), India. We thank B. S. Yadav and Sanjay Saxena for technical help.

References

Alexander MP (1969) Differential staining of aborted pollen and non-aborted pollen. Stain Technol 44:117–122

Arumugam N, Mukhopadhyay A, Gupta V, Pental D, Pradhan AK (1996) Synthesis of hexaploid (AABBCC) somatic hybrids: a bridging material for transfer of 'tour' cytoplasmic male sterility to different *Brassica species*. Theor Appl Genet 92:762–768

Arumugam N, Mukhopadhyay A, Gupta V, Pental D, Pradhan AK (2000) Somatic cell hybridization of 'oxy' CMS Brassica juncea (AABB) with B. oleracea (CC) for correction of chlorosis and transfer of novel organelle combinations to allotetraploid brassica. Theor Appl Genet 100:1043–1049

Arumuganathan K, Earle ED (1991) Estimation of nuclear DNA content of plants by flow cytometry. Plant Mol Biol Rep 9:229–241

Bannerrot H, Boulidard L, Couderon Y, Temple J (1974) Transfer of cytoplasmic male sterility from *Raphanus sativus* to *Brassica oleracea*. In: Wills AB, North C (eds) Proc Eucarpia Meet Cruciferae. Scottish Hortic Res Inst, Invergavrie, UK, pp 52–54

Budar F, Delourme R, Pelletier G (2004) Male sterility. In EC Pua, Douglas (eds) Biotechnology in agriculture and forestry 54. Springer, Berlin Heidelberg New York, pp 43–64

Delourme R, Renard M (1988) Cytoplasmic male sterility in rapeseed (*Brassica napus* L.): Female fertility of restored rapeseed with "Ogura" and cybrids cytoplasms. Genome 30:234–238

Delourme R, Eber F, Renard M (1991) Radish cytoplasmic male sterility in rapeseed: breeding restorer lines with a good female fertility. Proc 8th Int Rapeseed Congr 5:1506–1510

Fu TD (1981) Production of and research in rapeseed in the People's Republic of China. Eucarpia Cruciferae Newsl 6:6–7

Gourret J, Delourme R, Renard M (1992) Expression of *ogu* cytoplasmic male sterility in cybrids of *Brassica napus*. Theor Appl Genet 83:549–556

Havey MJ (2004) The use of cytoplasmic male sterility for hybrid seed production. In: Daniell H, Chase C (eds) Molecular biology and biotechnology of plant organelles. Springer, Dordrecht, The Netherlands, pp 617–628

Heslop-Harrison J, Heslop-Harrison Y, Shivanna KR (1984) The evaluation of pollen quality and further appraisal of the fluorochromatic (FCR) test procedure. Theor Appl Genet 67:367–375

Heyn FW (1976) Transfer of restorer genes from *Raphanus* to cytoplasmic male sterile *Brassica napus*. Eucarpia Cruciferae newsl 1:15–16

Kirti PB, Mohapatra T, Baldev A, Prakash S, Chopra VL (1995a) A stable cytoplasmic male sterile line of *Brassica juncea* carrying restructured organelle genomes from the somatic hybrid *Trachystoma ballii* + *Brassica jucea*. Plant Breed 114:434–438

Kirti PB, Banga SS, Prakash S, Chopra VL (1995b) Transfer of *ogu* cytoplasmic male sterility to *Brassica juncea* and improvement of the male sterile line through somatic cell fusion. Theor Appl Genet 91:517–521

Kirti PB, Baldev A, Gaikwad K, Bhat SR, Dinesh Kumar V, Prakash S, Chopra VL (1997) Introgression of a gene restoring fertility to CMS (*Trachystoma*) *Brassica juncea* and the genetics of restoration. Plant Breed 116:259–262

Liu Z, Guan C, Zhao F, Chen S (2005) Inheritance and mapping of a restorer gene for the rapeseed cytoplasmic male sterile line 681A. Plant Breed 124:5–8

Mollers C, Iqbal MCM, Roebbelen G (1994) Efficient production of doubled haploid *Brassica napus* plants by colchicine treatment of microspores. Euphytica 75:95–104

O'Brien TP, McCully ME (1981) The study of plant structure principles and selected methods. Termarcarphi Pty. Ltd., Melbourne Australia

Ogura H (1968) Studies of new male sterility in Japanese radish with special reference to the utilization of sterility towards the practical raising of hybrid seeds. Memoirs of the faculty of agriculture, Kagoshima University, Japan 6:39–48

Pelletier G, Primard C, Vedel F, Chetrit P, Remy R, Rousselle P, Renard M (1983) Intergeneric cytoplasmic hybridization in Cruciferae by protoplast fusion. Mol Gen Gent 191:244–250

Pradhan AK, Mukhopadhyay A, Pental D (1991) Identification of the putative cytoplasmic donor of a CMS system in *Brassica juncea*. Plant Breed 106:204–208

Pradhan AK, Sodhi YS, Mukhopadhyay A, Pental D (1993) Heterosis breeding in Indian mustard (*Brassica juncea* L. Czern & Coss): analysis of component characters contributing to heterosis for yield. Euphytica 69:219–229

Prakash S, Raut RN (1983) Artificial synthesis of *Brassica napus* and its prospects as an oilseed crop in India. Indian J Genet 43:282–290

Prakash S, Chopra VL (1990) Male sterility caused by cytoplasm of Brassica oxyrrhina in B. campestris and B. juncea. Theor Appl Genet 79:285–287

Prakash S, Kirti PB, Bhat SR, Gaikwad K, Dinesh Kumar V, Chopra VL (1998) A *Moricandia arvensis*-based cytoplasmic male sterility and fertility restoration system in *Brassica juncea*. Theor Appl Genet 97:488–492

Prakash S, Ahuja I, Upreti HC, Dinesh Kumar V, Bhat SR, Kirti PB, Chopra VL, (2001) Expression of male sterility in alloplasmic *Brassica juncea* with *Erucastrum canariense* cytoplasm and the development of a fertility restoration system. Plant Breed 120:479–482

Rao GV, Batra-Sarup V, Prakash S, Shivanna KR (1994) Development of a new cytoplasmic male-sterile system in *Brassica juncea* through wide hybridization. Plant Breed 112:171–174

Rogers SO, Bendich AJ (1994) Extraction of total cellular DNA from plants, algae and fungi. In: Gelvin S.V., Shilperoot R.A. (eds) Plant molecular biology mannual. Kluwer, Dordrecht, The Netherlands, pp 1–8

Sirivastava A, Gupta V, Pental D, Pradhan AK (2000) AFLP-based genetic diversity assessment amongst agronomically important natural and some newly synthesized lines of *Brassica juncea*. Theor Appl Genet 102:193–199