Indian J. Genet., 73(2): 203-210 (2013)
DOI : 10.5958/j.0975-6906.73.2.030

Short Communication

Transfer of cytoplasmic male sterility from alloplasmic *Brassica juncea* and *B. napus* to cauliflower (*B. oleracea* var. *botrytis*) through interspecific hybridization and embryo culture

R. Chamola, H. S. Balyan[1] and S. R. Bhat*

National Research Centre on Plant Biotechnology, Pusa Campus, New Delhi 110 012; [1]Department of Genetics and Plant Breeding, CCS University, Meerut 200 005

(Received: February 2013; Revised: March 2013; Accepted: April 2013)

Abstract

Cytoplasmic male sterile (CMS) lines of *Brassica juncea* and *B. napus* with the mitochondrial genome of *Moricandia arvensis* and *Erucastrum canariense*, respectively, were used to transfer CMS to cauliflower (*B. oleracea*). Embryo culture was essential to recover these interspecific hybrids. Success of embryo rescue was dependent on the post-pollination age of the ovary; ovaries excised at 9- and 14- days-after-pollination gave the best response in *B. napus* x *B. oleracea* and *B. juncea* x *B. oleracea*, respectively. Embryo rescue was also necessary in BC_1 and BC_2 to obtain progenies. Recovery of the recurrent parent phenotype was faster in *B. napus* x *B. oleracea* than *B. juncea* x *B. oleracea*. BC_3 generation plants of *B. napus* x *B. oleracea* showed good curd formation and complete male sterility and nine bivalents at meiosis whereas those of *B. juncea* x *B. oleracea* were male sterile but still had genetic elements of *B. juncea*.

Key words: *Brassica oleracea var. botrytis*, cauliflower, CMS, *Erucastrum canariense*, *Moricandia arvensis*

Cauliflower (*Brassica oleracea* var. *botrytis* L.) is the most popular Cruciferous vegetable. With an annual production of 5 M tons from a cropped area of 0.278 M ha, India is the largest producer and consumer of cauliflower in the world [1]. Cauliflower is a rich source of vitamin A and C, besides minerals such as phosphorus, potassium, calcium, sodium and iron. It also contains potent anti-cancer compounds such as diindolylmethane, sulforaphane and selenium [2, 3]. Cauliflower has shown marked heterosis for earliness, net weight of curd, and productivity, besides combining resistance to disease and abiotic stress [4]. In addition, hybrids provide uniformity of produce, a major quality parameter in cauliflower. Therefore, in cauliflower, cabbage and broccoli, hybrid breeding is widely followed.

Traditionally, hybrid seed production in cauliflower is based on self-incompatibility. However, self-incompatibility is genetically complex, often breaks down and poses problem in the maintenance of inbred lines [5]. Considering that cytoplasmic male sterility (CMS) could simplify hybrid seed production [6], efforts were made to obtain *B. oleracea* CMS lines using cytoplasms from closely related species such as *B. nigra* (L.) W.D.J. Koch [7], *B. napus* L. (*pol* cytoplasm) [8] and *B. rapa* ssp. *pekinensis* [9]. But poor stability of CMS and/or adverse effect on agronomic traits prevented their use in commercial hybrid seed production. Stable CMS lines were obtained with *Raphanus sativus* L. (male-sterility-inducing *ogura* cytoplasm) [10], *B. tournefortii* Gouan [11], *Diplotaxis muralis* (L.) DC [12] cytoplasms. However, chlorosis or floral deformities accompanied CMS with these cytoplasms. With further improvement involving somatic hybridization, commercially useful CMS lines

*Corresponding author's e-mail: srbhat@nrcpb.org
Published by Indian Society of Genetics & Plant Breeding, F2, First Floor, NASC Complex, PB#11312, IARI, New Delhi 110 012
Online management by indianjournals.com

have been derived from *ogura* cytoplasm [13]. Current CMS-based hybrids of cauliflower use this single source of cytoplasm and are therefore genetically vulnerable to biotic and abiotic stress. Recent availability of alloplasmic CMS lines of mustard carrying cytoplasm of *Moricandia arvensis* (L.) DC, *D. erucoides* (L.) DC, *Trachystoma ballii* O. E. Schulz, *Erucastrum canariense* Webb & Berthel [14] provides opportunity to develop new CMS lines of cauliflower and thus diversify CMS sources. The present study was aimed at transfer of male sterility inducing cytoplasms *E. canariense* or *M. arvensis* from alloplasmic lines of *B. napus* and *B. juncea* (L.) Czern & Coss. respectively to cauliflower through interspecific hybridization.

CMS (*M. arvensis*) *B. juncea* cv. RLM 198, CMS (*E. canariense*) *B. napus* and cauliflower (*B. oleracea* var. *botrytis*) cv. Pusa Meghna were used. Seeds of the CMS lines were sown in October whereas cauliflower was planted in August to achieve synchronization of flowering of these lines. Sterile anthers were removed from flower buds of CMS lines 1-3 days prior to anthesis and covered with butter paper bags. Freshly collected pollen from cauliflower was used to pollinate the emasculated buds the next day. The pollinated buds were covered with bags. Ovaries for *in vitro* culture were excised 5-, 9- and 14-days-after-pollination (DAP) and surface sterilized for 10 minutes with an aqueous solution containing 0.1% each of mercuric chloride and sodium dodecyl sulfate. After rinsing four times with sterilized distilled water, 3-4 ovaries were cultured per tube (150 x 25 mm) containing semi solid [15] (MS) medium supplemented with 30 gl^{-1} sucrose and 250 mg l^{-1} casein hydrolysate. The tubes were incubated at 25±2^{o}C under 16 h photoperiod (25 μE $m^{-2}s^{-1}$). After 15-20 days, ovaries were dissected and ovules were cultured for germination on a fresh MS medium of the same composition but without casein hydrolysate. The ovules that germinated were transferred to tubes containing MS medium with 0.2 mgl^{-1} benzylaminopurine for further shoot proliferation. Well-developed shoots were transferred to rooting medium (MS + 1 mg l^{-1} IBA) and incubated under same conditions as above. After four weeks, plantlets with good root development were removed from the medium, gently washed under running tap water to remove the traces of the medium, and placed for hardening for a week in tubes containing tap water such that only the root part was in water. The tubes were kept under plastic cover to achieve high humidity and incubated at 25±2^{o}C under 16 h photoperiod for 4-6 days. Hardened plants were transferred to pots filled with 2:1 mixture of soil: farm yard manure and grown in net house during October-March. Emasculation, pollination and ovary/embryo culture were repeated during successive backcross generations.

Flower buds for cytology were fixed in ethyl alcohol:chloroform:acetic acid (6:3:1 v/v). After 24 h, flower buds were shifted to 70% ethanol and kept at 5^{o}C till use. For chromosome count, anthers were hydrolyzed in 1N HCl at 60^{o}C for 10 min. and washed with distilled water to remove the traces of HCl. Washed anthers were transferred to Feulgen stain in the dark for 30 min. Stained anthers were squashed on a slide in a drop of acetocarmine (2%), a cover slip was placed, and pressed gently between folds of blotting paper with the thumb to spread the cells. Slides were examined under microscope and chromosome counts were made at diakinesis or late metaphase to early anaphase.

Initially, ca.300 buds of each cross were examined for seed set under natural conditions. Cross pollinated ovaries started yellowing and drying 10-15 DAP and dropped off without setting seed. With *B. napus* as the maternal parent, a few silique were formed but they were devoid of seeds. Signs of senescence of crossed flower buds appeared earlier (at 10-12 DAP) in *B. juncea* while in *B napus*, it was delayed till 20 DAP. Our results are in agreement with previous studies where very few hybrids were recovered from artificial cross pollination between *B. napus* and *B. oleracea* [16, 17] and until recently no spontaneous hybrid was reported under natural conditions [18]. Further, sexual hybrids between *B. juncea* and *B. oleracea* have been rarely reported [19] suggesting high incompatibility. Therefore, embryo rescue was resorted to obtain progenies.

Pistils harvested at 5-, 9- and 14-DAP were used for *in vitro* culture. Overall about 43% ovaries from the cross CMS (*M. arvensis*) *B. juncea* x *B. oleracea* remained green in culture. *In vitro* survival of ovaries varied with the time of sampling; ovaries harvested at 9 DAP remained green, increased in size and showed maximum (78.3%) survival after 15-20 days of *in vitro* culture (Fig. 1a). When such ovaries were split open, turgid, green or hard brown and pin-head sized ovules were recovered (Fig. 1b). Excised ovules were further cultured on the same medium. Maximum number of ovules (1.55 per ovary) were recovered from ovaries sampled 9 DAP. Of these, only 14 (19.17%) grew

further and gave rise to plants (Table 1). Survival of ovaries and recovery of ovules were low from ovaries harvested at 5- and 14-DAP and only two plants were recovered from these samples. In all, 8.9% seedlings could be obtained (Fig. 1c, d, e) from the cross CMS (*M. arvensis*) *B. juncea* x *B. oleracea*.

Table 1. Summary of results of embryo rescue on the recovery of interspecific hybrids between *B. juncea* or *B. napus* and *B. oleracea var. botrytis*

Cross	Time of sampling of ovaries	No. of ovaries cultured	No. of surviving ovaries	No. of ovules obtained and cultured	No. of plants recovered
CMS (*M. arvensis*) *B. juncea* x *B. oleracea*	5 DAP	60	12	11	-
	9 DAP	60	47	73	14
	14 DAP	60	19	15	2
CMS (*E. canariense*) *B. napus* x *B. oleracea*	5 DAP	60	0	0	0
	9 DAP	60	28	23	2
	14 DAP	60	32	47	5

The cross CMS (*E. canariense*) *B. napus* x *B. oleracea* also showed similar trend with respect to survival of ovaries and recovery of seedlings. Ovaries harvested at 5 DAP failed to survive. Maximum survival of ovaries and recovery of ovules was observed in pistils sampled at 14 DAP. Although ovaries cultured at 9 DAP remained green and gave about 0.8 ovules per ovary, only two plants were recovered. On per pollination basis, 3.9% seedlings could be obtained from this cross. Time of sampling of ovaries for *in vitro* culture has been found to be critical for success [20]. Our results show that *B. juncea* x *B. oleracea* hybrids can be obtained as readily as *B. napus* x *B. oleracea*. However, the optimum time of sampling of ovaries for *in vitro* culture was earlier (9 DAP) in *B. juncea* x *B. oleracea* than *B. napus* x *B. oleracea*.

Plants of the F_1 and backcross generations were male sterile and failed to set seed upon open pollination. Hence, sequential ovary and ovule culture, as per the optimum conditions determined earlier, was followed to obtain progeny generations. BC_2 plants showed some seed set upon open pollination.

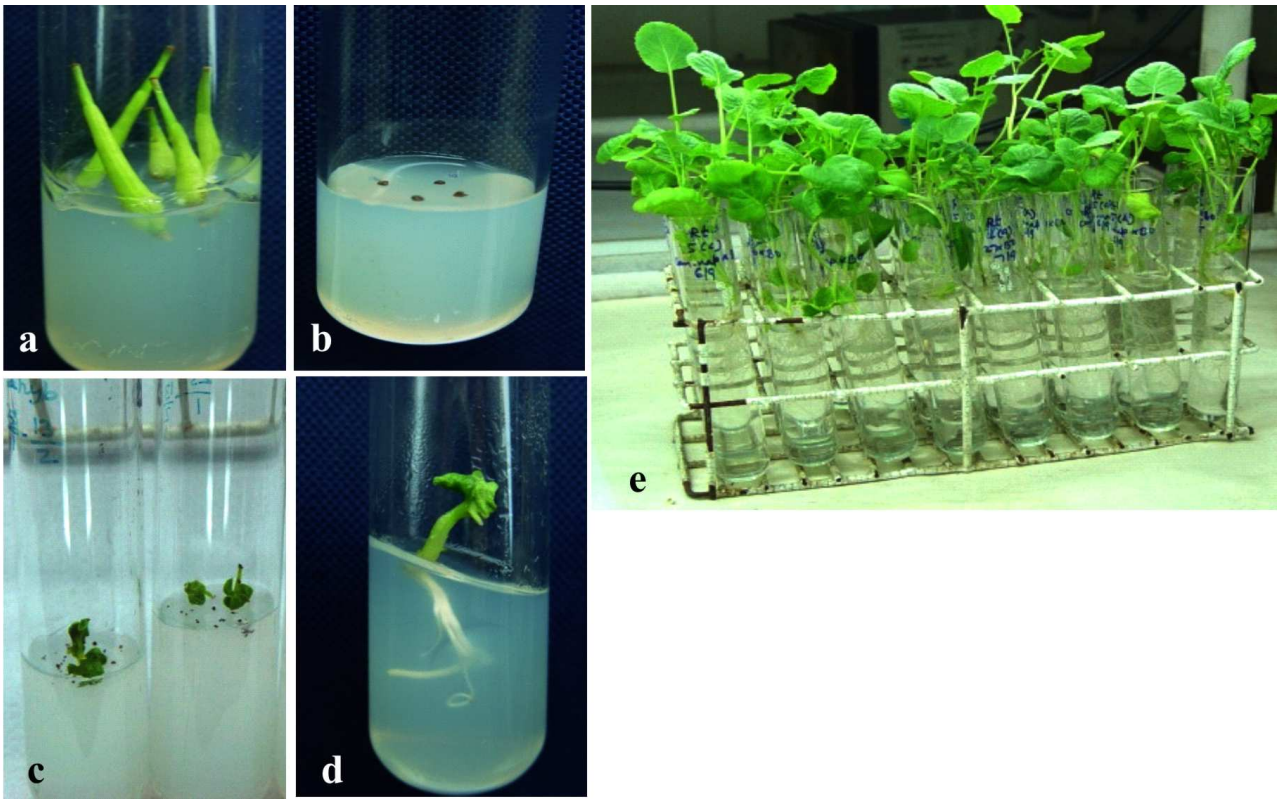

Fig. 1. Sequential ovary and embryo culture to obtain interspecific hybrids between *Brassica juncea/B. napus* and *B. oleracea*. a- ovary culture, b- dissected embryos from 20-day-old ovary culture, c- germinated embryos, d- young seedling, e- hybrid plants at hardening stage

Nevertheless, for advancing to BC_3, embryo rescue was followed. The success of embryo rescue improved during BC_2 generation with nearly every ovary cultured giving a plant. Plants obtained from embryo rescue were multiplied *in vitro*, hardened, transferred to pots, and raised in net house during the normal growing season (Oct- March).

Morphological features of the F_1 and back cross progenies

CMS (*M. arvensis*) *B. juncea* x *B. oleracea*: F_1 plants (Fig. 2a) obtained from embryo rescue were intermediate between the parents and thus were true hybrids. They were male and female sterile as expected of a plant with the ABC genome constitution. A few terminal, late-emerging flowers formed siliques but failed to set seed under open pollination. BC_1 generation plants had features of *B. oleracea* (Fig. 2b) but displayed a range of leaf and floral phenotypes with some displaying greater resemblance to *B. juncea* while others showing features of *B. carinata* or *B. oleracea*. The inflorescence had short branches that produced *B. juncea* like yellow flowers. A range of deformities was also observed in the BC_1. For example, a few BC_1 plants produced cream colored flowers with two pistils twisted together (Fig. 2d). Some plants remained dwarf, leafy and died without bolting.

BC_1 plants with greater resemblance to *B. oleracea* were chosen for further backcrossing. BC_2 generation plants also showed a wide variety of phenotypes, male and female sterility. Some BC_2 generation plants also set a few seeds upon open pollination. In BC_3 generation, cauliflower-like plants were recovered. These plants had distinct features of cauliflower, particularly with respect to leaf and curd morphology (Fig. 2e). These plants were short, grew slowly and produced small green curd that soon elongated to produce profuse inflorescence branches (Fig. 2c). They came to flowering around the same time as *B. oleracea*. The flowers were completely male sterile (Fig. 2f). Cytological preparation of meiocytes showed 2 multivalents, 7 bivalents and 4 pairs of univalents migrating towards poles at the late metaphase I stage (Fig. 2g) indicating the presence of *B. juncea* chromosomes.

CMS (E. canariense) B. napus x B. oleracea var. botrytis

Fewer hybrids were recovered from this cross as compared to the cross CMS (*M. arvensis*) *B. juncea* x *B. oleracea.* However, in subsequent cycles of backcrossing, success of embryo rescue improved. Under open pollination, BC_1 and BC_2 plants set siliques but seed formation did not occur in BC_1 whereas in BC_2, each silique on average bore at least one seed. F_1 hybrids were morphologically similar to the female parent *B. napus* (Fig. 3a) but the inflorescence was intermediate between *B. napus* and *B. oleracea.* BC_1 plants resembled *B. oleracea* for leaf, curd and general morphology (Fig. 3b, c). However, the curd was small and green. In BC_2 generation, the curd size improved (Fig. 3d) but floral deformities were still present. BC_3 plants had large, thick, waxy, elongated and bluish-green leaves resembling greatly the leaves of cauliflower (Fig 3f). One of the BC_3 plants produced white, compact curd (Fig. 3e) and later turned creamy. It bore yellow, male sterile flowers (Fig. 3g). Ovaries were large, healthy and normal. A good seed set was found upon pollination indicating normal female fertility. Cytological preparation of meiocytes showed 9 II in the PMCs of BC_2 plant (Fig. 3h).

There are several reports of transfer of genes governing traits such as Triazine-herbicide tolerance [21, 22], self-incompatibility [23, 24], cabbage aphid resistance [25] between *B. oleracea* and *B. napus*. In contrast, only a few studies are available on gene transfer between *B. juncea* and *B. oleracea*. Fusion of protoplasts of CMS (*B. oxyrrhina*) *B. juncea* and *B. oleracea* was done to obtain CMS lines with substituted *B. oxyrrhina* plastids [26]. Similalrly, somatic hybridization between leaf mustard (*B. juncea*) and broccoli (*B. oleracea*) was employed to transfer CMS and Verticillium wilt resistance from *B. oleracea* to *B. juncea* [27]. The present study demonstrates that both *B. juncea* and *B. napus* could serve as donors of both cytoplasmic as well as nuclear genes for the improvement of *B. oleracea*. In the era of transgenics, there is concern about gene flow between transgenic crops and natural feral populations. For instance, it was found that *B. oleracea* and *B. napus* can give rise to hybrids in nature albeit at very low frequency [18]. The range of phenotypes found in backcross populations in this study indicate that such natural hybrids could succeed in producing progenies and thereby contribute to gene flow.

Our results also show that alloplasms of *E. canariense* and *M. arvensis* impart cytoplasmic male sterility to *B. oleracea*. Further, *E. canariense* plastids did not cause leaf chlorosis in the *B. oleracea* nuclear background indicating nuclear-plastid compatibility. The recovery of cauliflower phenotype required at least

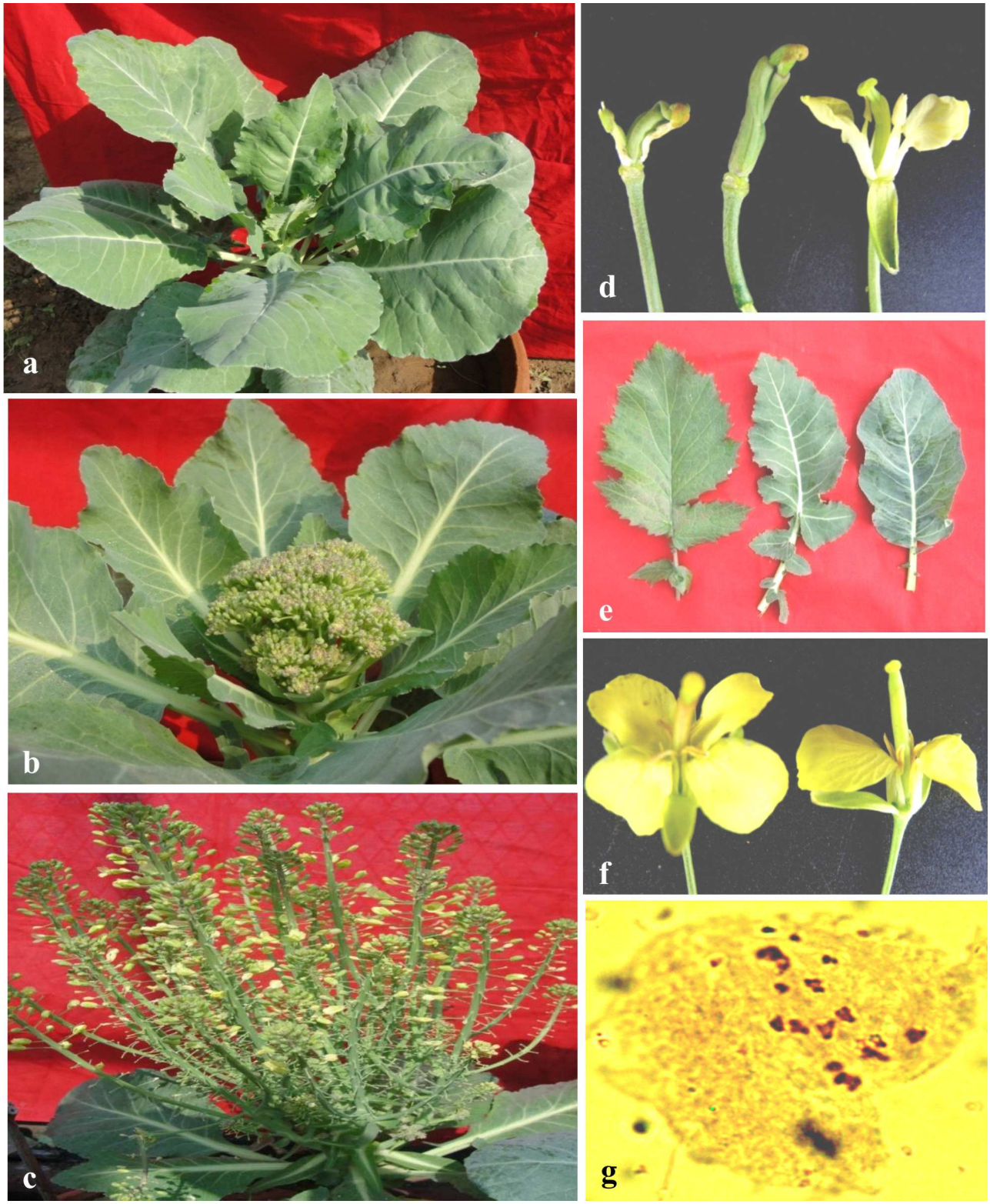

Fig. 2. Interspecific hybridization between CMS (*M. arvensis*) *B. juncea* x *B. oleracea*. a to c- F_1, BC_1 and BC_2 plants of *B. juncea* x *B. oleracea*; d- crooked and fused pistils of BC_1 flowers; e- leaves of CMS *B. juncea* (left), BC_3 (centre) and *B. oleracea* (right); f- male sterile flowers of BC_3 plant; g- Meiotic preparation of BC_2 plant at the late metaphase I stage showing 2 multivalents, 7 bivalents and 4 pairs of univalents migrating towards poles

Fig. 3. Interspecific hybridization between CMS (*E. canariense*) *B. napus* x *B. oleracea*. a – F_1; b - leaves of CMS *B. napus* (left), BC_1 (centre) and *B. oleracea* (right); c to e– curd formation in BC_1, BC_2, and BC_3; f - leaves of CMS *B. napus* (left), BC_3 (centre) and *B. oleracea* (right); g - inflorescence in BC3 of *CMS (E. canariense) B. oleracea*; h- Meiotic preparation of BC_2 plant at the late metaphase I stage showing 9II

three back cross of the interspecific hybrids to the recurrent cauliflower parent. Despite presence of 2n=20 chromosome number, BC_3 plants did not completely recover cauliflower phenotype suggesting homeologous recombination between *B. oleracea* and *B. rapa/B. nigra* chromosomes in the F_1 and backcross generation leading to persistence of introgressed genes. Allosyndetic pairing between A, B and C genome chromosomes have been reported in interspecific crosses of Brassica [28]. Recently, Wen et al. [29] reported greater frequency of allosyndetic pairing of B genome chromosomes in ABCC hybrids than AABCC hybrids which resulted in faster loss of B genome chromosomes in progenies of the later hybrids. Similar events might have contributed to slower recovery of cauliflower genome in these interspecific hybrids.

In conclusion, the present study demonstrated that embryo rescue could be used to transfer genes from *B. napus/B. juncea* to *B. oleracea*, and both *E. canariense* and *M. arvensis* mitochondrial genomes confer CMS in *B. oleracea*. The CMS lines generated show no abnormalities and could be used for heterosis breeding in cauliflower.

References

1. **FAOSTAT.** 2010. Food and Agriculture Organization of the United Nations.
2. **Lampe J.W. and Peterson S.** 2002. Brassica, biotransformation and cancer risk: genetic polymorphisms alter the preventive effects of cruciferous vegetables. J. Nutr., **132**: 2991-2994.
3. **Brandi G., Schiavano G. F., Zaffaroni N., Macro C. D., Paiardini M., Cervasi B. and Maguani M.** 2005. Mechanisms of action and antiproliferative properties of *Brassica oleracea* juice in human breast cancer cell lines. J. Nutr., **135**: 1503-1509.
4. **Zhiyaun F., Sun P., Liu Y., Yang L., Wang X., Hou A. and Bian C.** 1997. A male sterile line with dominant gene (Ms) in cabbage (*Brassica oleracea* var. *capitata*). J. South-west Agril. University, **3**: 265-268.
5. **Kucera V., Chytilová V., Vyvadilová M. and Klíma M.** 2006. Hybrid breeding of cauliflower using self-incompatibility and cytoplasmic male sterility. Hort. Sci. (Prague), **33**: 148-152.
6. **Renard M., Delourme R, Mesquida J, Pelletier G, Primard C, Boulidard L, Dore C., Ruffio V., Morice J.** 1992. Male sterilities and F_1 hybrids in Brassica. *In*: Datte Y., Dumas C., Gallais A. (eds.) Reproductive Biology and Plant Breeding. Springer, Berlin Heidelberg, New York, pp. 107-119.
7. **Pearson O. H.** 1972. Cytoplasmically inherited male sterility characters and flavor components from the species cross *Brassica nigra* (L.) Koch x *B. oleracea* L., J. Amer. Soc. Hort. Sci., **97**: 397-402.
8. **Shiga T. and Baba S.** 1973. Cytoplasmic male sterility in oilseed rape (*Brassica napus* L.) and its utilisation to breeding. Japan J. Breed., **23:** 187-193.
9. **Zhu P. and Wei Y.** 2009. Compatibility, production of interspecific F1 and BC1 between improved CMS *Brassica campestris* ssp. *pekinensis* and *B. oleracea* var. *acephala*. J. Plant Breeding and Crop Sci., **1**: 265-269.
10. **Bannerot H., Boulidard L., Cauderon Y. and Tempe J.** 1974. Transfer of cytoplasmic male sterility from *Raphanus sativus* to *Brassica oleracea*. Proc. EUCARPIA Meeting-Cruciferae, 1974, 52-54.
11. **Cardi T. and Earle E. D.** 1997. Production of a new CMS Brassica oleracea by transfer of Anand cytoplasm from *Brassica rapa* through protoplast fusion. Theor. Appl. Genet., **94**: 202-212.
12. **Shinada T., Kikuchi Y., Fujimoto R. and Kishitani S.** 2006. An alloplasmic male-sterile line of *Brassica oleracea* harboring the mitochondria from *Diplotaxis muralis* expresses a novel chimeric open reading frame, orf72. Plant Cell Physiol., **47**: 549-553.
13. **Pelletier G., Primard C., Vedel F., Chetrit P., Remy R., Roussele P. and Renard M.** 1983. Intergeneric cytoplasmic hybridization in Cruciferae by protoplast fusion. Mol. Gen. Genet., **191:** 244-250.
14. **Prakash S., Bhat S. R. and Fu T. D.** 2009. Wild germplasm and male sterility. In Biology and Breeding of Crucifers, pp 113-127. Edt. S K Gupta. Publishers CRC Press, Taylor & Francis Group, NY, USA.
15. **Murashige T. and Skoog F.** 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant., **15**: 473-497.
16. **Honma S. and Summers W. L.** 1976. Interspecific hybridization between *Brassica napus* L. (Napobrassica group) and *B. oleracea* L. (Botrytis group). J. Am. Soc. Hort. Sci., **101**: 299-302.
17. **Chiang M. S., Chiang B. Y. and Grant W. F.** 1977. Transfer of resistance to race 2 of *Plasmodiophora brassicae* from *Brassica napus* to cabbage (*Brassica oleracea* var.*capitata*). I Interspecific hybridization between *B. napus* and *B. oleracea* var. *capitata*. Euphytica, **26:** 319-326.
18. **Ford C. S., Allainguillaume J., Grilli-Chantler P., Cuccato G., Allender C. J. and Wilkinson M. J.** 2006. Spontaneous gene flow from rapeseed (*Brassica napus*) to wild *Brassica oleracea*. Proc. R. Soc. B **273**: 3111-3115. doi:10.1098/rspb.2006.3686
19. **Busso C., Attia T. and Röbbelen G.** 1987. Trigenomic combinations for the analysis of meiotic control in the cultivated Brassica species. Genome, **29**: 331-333.

20. **Wen, J., Tu, J. X., Li Z. Y., Fu T. D., Ma C. Z. and Shen J. X.** 2008. Improving ovary and embryo culture techniques for efficient resynthesis of *Brassica napus* from reciprocal crosses between yellow-seeded diploids *B. rapa* and *B. oleracea*. Euphytica, **162**: 81-89.

21. **Ayotte R., Harney P. M. and Souza Machado V.** 1987. Transfer of triazine resistance from *B. napus* L. to *B. oleracea* L. I. Prduction of F1 hybrids through embryo rescue. Euphytica, **36**: 615-624.

22. **Ayotte R., Harney P. M. and Souza Machado V.** 1988. Transfer of triazine resistance from *B. napus* L to *B. oleracea* L. III. First backcross to parental species. Euphytica, **37**: 189-197.

23. **Repley V. L. and Beversdorf W. D.** 2003. Development of self incompatible *Brassica napus* : (I) introgression of S-allele from *Brassica oleracea* through interspecific hybridization. Plant Breed., **122**: 1-5.

24. **Rahman M. H.** 2005. Resynthesis of *Brassica napus* L. for self-incompatibility: self-incompatibility reaction inheritance and breeding potential. Plant Breed., **124**: 13-19.

25. **Quazi M. H.** 1998. Interspecific hybrids between *Brassica napus* L. and *Brassica oleracea* L. developed by embryo culture. Theor. Appl. Genet., **75:** 309-318.

26. **Verma J. K., Mukhopadhyay A., Arumugam N., Sodhi Y. S., Pradhan A. K., Pental D. and Gupta V.** 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 brassicas. Theor. Appl. Genet., **100**: 1043-1049.

27. **Lian Y. J., Lin G. Z., Zhao X. M. and Lim H. T.** 2011. Production and genetic characterization of somatic hybrids between leaf mustard (*Brassica juncea*) and broccoli (*Brassica oleracea*). *In vitro* Cell. Dev. Biol. Plant, **47**: 289-296.

28. **Mason A. S., Huteau V., Eber F., Coriton O., Yang G., Nelson M. N. Cowling W. A. and Chevre A. M**. 2010. Genome structure affects the rate of autosyndesis and allosyndesis in AABC, BBAC and CCAB Brassica interspecific hybrids. Chromosome Res., **18**: 655-666.

29. **Wen J., Lixia Z., Liping Q., Hongmei K., Bin Y., Jinxiong S., Jinxing T., Chaozhi M. and Fu T. D.** 2012.Characterization of interploid hybrids from crosses between *Brassica juncea* and *B. oleracea* and the production of yellow-seeded *B. napus*. Theor. Appl. Genet., **125**: 19-32.