CYTOPLASMIC MALE STERILITY (CMS) SYSTEMS OTHER THAN OGU and POLIMA IN BRASSICAE: CURRENT STATUS

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Considerable effort has been invested in the development of male sterility in Brassicas which has resulted in the two widely exploited cytoplasm induced male sterility system (CMS) known as Ogura and Polima. The need exists still developing new and better types, because some of the cytoplasms are associated with diseases and floral abnormalities. Brassica coenospecies, a group corresponding to subtribe Brassicinae of Crucifer family is a rich repository of germplasm not only for nuclear but also for plasma genes (Warwick & Black, 1991; Pradhan et al 1992). Majority of the members of this coenospecies have close homoeology with crop species. It is now established that alloplasmics - the combination of nucleus and alien cytoplasm often express male sterility. Wide hybridization, therefore is a relevant procedure for developing CMS. Alloplasmics have been produced either by sexual hybridization or by protoplast fusion. A major difference is that in the former the uniparental cytoplasm remains unaltered while in the latter, organelle assortment and mitochondrial (mt) DNA recombination are of frequent occurrence. Synthesis of CMS B.oleracea var. italica (broccoli) carrying B.nigra cytoplasm (Pearson, 1972) and of B.campestris var. Yukina (a leafy type) with Diplotaxis muralis cytoplasm (Hinata & Konno, 1979) was the pioneering work. Since then a number of CMS systems have been developed, majority of these are from our laboratory(*). These are briefly reviewed here:

Brassica nigra(nig) system

Developed initially by Pearson (1972) in B.oleracea from the synthetic alloploid B.nigra x B.oleracea, it has been successfully transferred to cabbage and cauliflower. The CMS plants are normal, but two types of flowers were observed: (1) with petaloid anthers and without nectaries, and (2) with rudimentary anthers. Seed set was initially poor, however, Dickson & Kyle (1987) selected some lines with improved seed set. B.campestris chloroplasts encoding atrizine resistance (ATR) were introgressed to CMS (nig) broccoli through protoplast fusion (Christey et al 1991) and it was also suggested that ATR CMS plants could be used in hybrid seed production to eliminate pollinations after fertilization.

Diplotaxis muralis (mur) system

This CMS was obtained in B.campestris by Hinata & Konno (1979) from the synthetic alloploid D.muralis x B.campestris var. Yukina (2n=62). The CMS plants are normal green, but the flowers have narrow petals. Anthers are short and occasionally petaloid. Pollen production is also drastically reduced. The flowers have only two out of the expected four nectaries and almost no nectar is produced. When this cytoplasm was introgressed to B.napus (Pellan- Delourme & Renard, 1985) it was observed that majority of B.napus accessions possessed restoring genes while only few were maintainers. Sterility in B.napus is stable but seed set is low. Fan et al (1985) also observed that all the European B.napus accessions were restorers for mur cytoplasm. They also reported that an extra chromosome of D.muralis is responsible for sterility in backcross progeny. Restorers for mur cytoplasm are available in B.campestris and B.napus.

B.tournefortii (tour) system

Rawat and Anand (1979) reported a CMS of spontaneous origin in B.juncea. Later the sterility was attributed to alloplasmic interaction. Substantiating evidence for the latter proposal has come from comparison of cp DNA profiles of this CMS and a number of species in Brassica coenospecies. It is now established that this CMS is not due to a mutation but is alloplasmic, B.tournefortii being the cytoplasmic donor (Pradhan et al 1991). It seems to originate from the synthetic alloploid B.tournefortii x B.nigra originally produced by Narain & Prakash (1972). Stiewe et al (1995) isolated CMS (tour) B.napus from the somatic hybrid B.tournefortii + B.napus. The CMS plants have recombined mt and the chloroplasts were contributed by B.napus. Floral abnormalities were frequently observed in CMS B.juncea which included crooked style, rudimentary and petaloid anthers and reduced nectaries. Female fertility is also very low (41%) and the plants are highly susceptible to white rust. However, in B.napus this CMS does not cause drastic floral abnormalities and the female fertility is also normal. Restorer genes were located in natural accessions of B.napus (Sodhi et al 1994; Banga et al 1995) and also transferred from B.tournefortii (Stiewe 1995).

Brassica oxyrrhina (oxy) system*

Male sterility system, based on B.oxyrrhina cytoplasm, has been developed in B.campestris, B.juncea and B.napus following the synthesis of alloploid B.oxyrrhina x B.campestris (2n=38,00AA) and its repeated backcrossings to the crop species (Prakash and Chopra, 1990). Male sterile plants have delayed flowering and the leaves are chlorotic. The degree of chlorosis varies - from severe (B.campestris) to mild (B.napus). Flowers have normal appearance, excellent nectaries and slender indehiscent anthers. Meiosis in all the three alloplasmics is normal with regular bivalent occurrence. Pollen abortion occurred after tetrad formation. Female fertility is absolutely normal (94-96%). Chlorosis correction in B.juncea has been achieved through protoplast fusion (Kirti et al 1993). Restorers for this CMS are so far not available

Diplotaxis siifolia (siifolia) system

TheD.siifolia system was obtained by Rao et al (1994)in B.juncea from the synthetic intergeneric alloploid Diplotaxis siifolia x B.juncea (2n=56, D^SD^S AABB). Alloplasmic plants are similar to normal parents but flowering is delayed by about 8 days. Flowers have normal nectaries but reduced sepals, petals and non-dehiscing anthers. Meiosis proceeds normally upto tetrad formation when degeneration of microspore starts still enclosed by the callose wall of the tetrad or soon after the dissolution of callose wall. Female fertility is comparable to normal fertile B.juncea. This sterility inducing cytoplasm has now been transferred to B.napus also. Restorers are so far not available.

Trachystoma ballii (trachy) system*

This CMS has been derivedin B.juncea from the somatic hybrid Trachystoma ballii + B.juncea (2n=52, TTAABB)(Kirti et al 1995). Male steriles, although similar to normal fertile plants in general morphology are 6-7 days late in flowering. Flowers have linear petals and the stamens turn into petal-like structures with a slender filament. Petaloid structures contain one or two small lateral sacs similar to anther locules and are full of sterile pollen grains. These structures do not dehisce and the number of sterile pollen grains is also drastically reduced. On pollination with B.juncea a seed set of 93% is obtained. Molecular analysis of the cytoplasm has revealed that it contains recombined mitochondria and chloroplast of Trachystoma ballii. This cytoplasm has been transferred to B.napus . Restorers are so far not available.

Moricandia arvensis (mori) system*

This system in B.juncea originated from the somatic hybrid Moricandia arvensis + B.juncea (2n=64, MM,AABB). Plants are slow growing, very late in flowering (30-35 days) and highly chlorotic, the leaves being almost yellow. Flowers have small sized, slender, non-dehiscent anthers and good nectaries. Female fertility is around 89%. A preliminary analysis of cytoplasmic organelles indicates that in the sterile plant, the mitochondria and chloroplasts are from the wild parent M.arvensis. This cytoplasm has been introgressed to B.napus also. Chlorosis correction in B.juncea has been achieved following protoplast fusion. Restorers are not available so far.

Diplotaxis catholica (catholica) system*

Developed in B.juncea, this system has been derived from the somatic hybrid D.catholica + B.juncea (2n=54, D^CD^CAABB) which had recombined mitochondria and B.juncea chloroplasts. CMS plants are normal green and vigorous but slightly late in flowering.Flowers closely resemble normal fertile flowers with excellent nectaries except the anthers are slender, smaller in size and non-dehiscent. Female fertility is absolutely normal.

Sinapis alba (alba) system*

CMS B.juncea plants carrying S.alba cytoplasm were obtained from the somatic hybrid S.alba + B.juncea (2n=60, $S^{al}S^{al}$ AABB). The plants are late in flowering (by 15 days), and normal green. Flowers have small sized non-dehiscing anthers, but nectaries are well developed. A preliminary molecular analysis of the cytoplasm reveals that both the mt and cp genomes derive from S.alba.

A general observation on these CMS is that the presence of alien cytoplasm usually delays the flowering . Another feature is mild to severe leaf chlorosis (oxy and mori). Floral abnormalities were frequently observed which range from narrow linear petals (siifolia, nig), petaloid anthers (nig, trachy, tour), vestigial rudimentary anthers (nig, siifolia, underdeveloped nectaries (tour, nig) and crooked style and contorted fruits (tour). These characters in combination with low bee visitations severely effect the female fertility in some CMS which has been observed as low as 41% in (tour) B.juncea.

A preliminary survey of meiotic process in these CMS has revealed that meiosis proceed normally. Pollen abortion occurs after tetrad formation just prior to microgametogenesis.

Protoplast fusion methodology has greatly helped the manipulation of cytoplasmic encoded traits for the improvement of existing CMS particularly in flower characteristics. A distinct advantage with this technique is that mt and cp encoded characters of the two parents can be combined through cell fusion which otherwise not possible by other in vitro method or sexual crossing because of maternal inheritance. Thus new combinations of cp and mt have been obtained. Elimination of leaf chlorosis in CMS (oxy) B.juncea was achieved and attributed to intergenomic chloroplast recombination (Kirti et al 1993) . Christey et al (1991) introgressed the chloroplast encoded atrizine resistance of B.campestris to (nig) B.oleracea broccoli. Pradhan et al (1995) generated rearranged/recombined mt DNA of varying degree in CMS (tour) B.juncea by fusing the protoplast of CMS plants and normal B.oleracea and male steriles with normal flowers were obtained.

It has been observed that CMS which have originated from somatic hybrids have in many instances recombinant mt DNAs. Such plants have less pronounced flower abnormalities , better nectaries, and normal female fertility. Protoplast fusion can also render the search for restorers much easier. The experiment of Stiewe et al (1995) elegantly demonstrates it when following B.napus and B.tournefortii protoplast fusion,they obtained a CMS (tour) B.napus with altered mitochondria and B.napus chloroplasts.Also a restorer line was developed by transferring relevant genes to B.napus from B.tournefortii. The emphasis on cell fusion technology for exploiting heterospecific cytoplasm is well placed because it aprovides opportunities of reassortment of cytoplasmic organelles but caution is prudent in execution. Malformation of flowers, distortions in fruit morphology and reduced seed set are met with occassionally and these have to conciously avoided.

Progress towards production of commercial hybrids based on these systems has been hampered primarily due to lack of proper restorers or stable maintainers. Non- availability of restorers could be attributed to multi-locus incompatibility between alien cytoplasm and crop nuclei. The extent of incompatibility can be reduced by generating mitochondrial recombinants. The ideal situation would be where a major part of alien mt genome is replaced by the crop mt genome but the sterility encoding regions are retained.

Although all the components of hybrid production are available with 'mur'CMS in B.napus and B.campestris no hybrid has been developed. On the other hand, limited success has been achieved with 'tour' CMS and a hybrid variety in B.napus PGSH-51 was recently released in India which has 20% yield advantage(Banga et al 1995) In rest of the cases, the search for restorers is continuing.

Conclusion

The cases reviewed in this paper suggest that wide hybridization is a potent tool for synthesizing alloplasmics of variable cytoplasmic origin as Brassica coenospecies provides a vast base. Extensive cytoplasmic heterogeniety can be generated through protoplast fusion.

In alloplasmic male sterility systems, the restorer gene/s are available in the cytoplasmic donor. These can be accessed through chromosomal manipulations either by developing chromosome addition lines or creating a situation where homoeologous recombination between the crop and cytoplasm donor chromosome will allow introgression.

REFERENCES

Kirti, P.B., Narasimhulu, S.B., Mohapatra, T., Prakash, S. and Chopra, V.L. (1993). Correction of chlorophyll deficiency in alloplasmic male sterile Brassica juncea through recombination between chloroplast genomes. Genetical Research Cambridge, 62: 11-14.

Kirti, P.B., Mohapatra, T., Prakash, S. and Chopra, V.L. (1995) Development of a stable cytoplasmic male sterile line of Brassica juncea from the somatic hybrid Trachystoma ballii + B.juncea . Plant Breeding Press)

Hinata, K. and Konno, N. (1979). Studies on a male sterile strain having the Brassica campestris nucleus and the Diplotaxis muralis cytoplasm. I. On the breeding procedure and some characteristics of male sterile strain. Japan Journal of Breeding. 29: 305-311.

Pearson, O.H. (1972). Cytoplasmically inherited male sterility characters and flavor components from the species cross Brassica nigra (L). Koch x B.oleracea L. Journal of American Society of Horticultural Science 97: 397-402

Prakash, S. and Chopra, V.L. (1990). Male sterility caused by cytoplasm of Brassica oxyrrhina in B.campestris and B.juncea.Theoretical and Applied Genetics, 79: 285-287.

Rao, G.U., Sarup, V.B., Prakash, S. and Shivanna, K.R (1994). Development of a new cytoplasmic male sterility system in Brassica juncea through wide hybridization.Plant Breeding,112: 171-174.

Rawat, D.S., and Anand, I.J. (1979). Male sterility in Indian mustard Indian Journal of Genetics and Plant Breeding, 39: 412-414.