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Research Article

Cytoplasmic male sterile systems in pigeonpea with special reference to A_7 CMS

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Abstract
Compared to the cereals and vegetable crops, cytoplasmic male sterility is comparatively a new system available to pigeonpea breeders to exploit heterosis/hybrid vigor. It has been possible to develop a CMS system for pigeonpea as cross pollination is possible in this crop. Various CMS systems have been developed for pigeonpea utilizing different wild relatives from different gene pools. CMS based hybrids developed from A_4 system have recorded superiority in yields compared to the checks on farmer's fields. This has opened up a new window for yield improvement in pigeonpea. The paper discusses different CMS systems available for pigeonpea in general and the A_7 system which was developed utilizing *Cajanus platycarpus*, in particular.

Keywords
Pigenopea, male sterile system, hybrid

Introduction

Spectacular progress in yield in many cereals, vegetables and a few food crops is attributed to the exploitation of hybrid vigour. To achieve a similar breakthrough in leguminous crops in general and pigeonpea (*Cajanus cajan* L. Millsp) in particular, such hybrid vigour need to be exploited. Many of the leguminous crops have self pollination mechanism to generate seeds and it is not favorable for hybrid seed production. Two exceptional food legumes which have a cross pollination mechanisms are pigeonpea and faba beans (*Vicia faba)* (Bond et al., 1966) thus opening avenues for the development of commercial hybrids.

A cytoplasmic-nuclear male-sterility (CMS) system was essential for pigeonpea as its productivity was stagnant at 700 kg/ha for decades in spite of more than 50 variety releases. In India, the annual pigeonpea grain production of 2.74 million tones falls short of domestic demand and about 0.5 to 0.6 million tones is imported from various countries of Asia and Africa. Therefore in order to meet the ever growing demand of pigeonpea, its productivity should be increased by adopting new and appropriate technologies. The climate change scenario has further enhanced the urgency to look for new sources/ technologies/ opportunities in hand to meet the urgent needs. Apart from this, pigeonpea farmers save seeds year after year for next year's planting. Due to out crossing nature of the crop there is tremendous variation in the seed for many of the economically important traits.

The first report on natural out crossing in pigeonpea was published by Howard *et al* in 1919. The phenomenon is not desirable in maintaining the genetic purity of cultivars in pigeonpea breeding as it creates variation leading to inconsistent yields and breakdown of disease resistance. However, this trait was utilized to develop hybrid breeding technology. To achieve this, development of CMS systems is essential. So far, seven cytoplasmic male sterile systems (CMS) have been reported for pigeonpea (Saxena *et al*., 2010). The systems offer ample opportunities to exploit heterosis in pigeonpea. The expression of cytoplasmic nuclear male sterility is controlled by genetic factor carried through the female parent, hence never lost or diluted during succeeding generations of reproduction. For a successful hybrid program, three genotypes are essential. The A line (male sterile line) with male sterile factors (S) in its cytoplasm has homozygous recessive nuclear genes for male sterility. The B line (maintainer) must have normal cytoplasm and homozygous recessive male sterility nuclear genes. The R line has nuclear genome with fertility restoration genes.

So far, seven CMS systems have been reported in pigeonpea (Saxena *et al*., 2010). Of these, six have been developed utilizing wild relatives from secondary gene pool of pigeonpea. One system has cultivated pigeonpea cytoplasm (Mallikarjuna and Saxena, 2005). The seventh system was developed utilizing *C. platycarpus*, a wild relative from tertiary gene pool of pigeonpea (Saxena et al., 2010; Mallikarjuna et al., 2011). A brief description of A_1 to A_6 is presented below with a detailed description of A_7.

A_1 CMS system with *C. sericeus* cytoplasm: A_1 CMS system was first reported by Ariyanayagam

et al., (1993). *C. sericeus* was used as the female parent in the crossing program and partial male sterile progenies were observed in the first generation with complete male sterile plants in F_2 generation. To stabilize the CMS trait, Ariyanayagam *et al*., (1995) later used multiple genome transfer method. Although male sterile progenies were obtained, Saxena and others made more crosses and developed CMS lines such as CMS 85010 A, CMS 88034A and CMS 13091A (Saxena *et al*., 2010). One of the undesirable traits of this system is temperature sensitivity. Male sterile lines revert back to fertility under low temperatures and again regain male sterility under high temperatures, a trait unfavorable to exploit heterosis. The system is not in wide use.

A_2 CMS system with *C. scarabaeoides* cytoplasm**:** The first report on the development of CMS from *C. scarabaeoides* was by Ariyanayagam *et al*., (1993). F_1 plants were partially male sterile and in the backcrosses, male sterile plants were obtained. Tikka *et al.*, (1997) obtained CMS plants and also their maintainers. This male sterile source was used to develop hybrids. Saxena and Kumar (2003) developed male sterile plants and identified many sources of maintainers and restorers. The CMS source was found to be unstable with large variation in fertility restoration. Later, fertility restoration was found among F_2 segregants and perfect male sterile maintainer line was also identified (Saxena *et al*., 2010)

A_3 CMS system with *C. volubilis* cytoplasm**:** CMS system developed utilizing *C. volubilis* cytoplasm, was called A_3 (Wanjari et al., 1999). It was not possible to obtain good fertility restorers for this system and hence was not used.

A_4 CMS system with *C. cajanifolius* cytoplasm**:** This CMS system was developed by using *C. cajanifolius*, a wild relative closest to cultivated pigeonpea and differing by 5-6 genes (Mallikarjuna et al., 2011b). Male sterile plants did not show any morphological deformity and produced plenty of pollen in the hybrid, in combination with restorers. The pollen fertility of fertility restorers was also good (Saxena et al., 2005). Evaluation of test hybrids developed from this source produced greater yield than the controls. Yield advantages to the proportion of 70-80% were obtained over the checks. The hybrids had acceptable seed size and produced pods in high numbers. The estimates of productivity per unit of time indicated that the yield was twice that of the control (Saxena et al., 2005).

A_5 CMS system with *C. cajan* cytoplasm**:** The first report of developing a CMS system with cultivated pigeonpea cytoplasm was from Mallikarjuna and Saxena (2005). Cultivated pigeonpea lines ICPL 85030, ICPL 85010, ICPL 88014 produced a few progenies with 100% male sterility. Anthesis in these lines was interrupted at pre as well as post meiotic stages (Mallikarjuna and Kalpana, 2004). Many pigeonpea cultivars restored fertility indicating that it might be a good source to exploit heterosis. Initially male sterility was maintained by the wild species parent. Later, HPL 24 produced both fertile and male sterile plants, suggesting that the presence of both fr and Fr genes in its nuclear genome. More recently, many of the fertile siblings have been found to maintain male sterility. Backcrossing male sterile lines with their fertile siblings have resulted in maintenance of male sterility (Mallikarjuna N, unpublished data). With the availability of maintainers of male sterility and restorers of fertility, the A_5 system can be used to exploit heterosis in pigeonpea.

A_6 CMS system with *C. lineatus* cytoplasm**:** A naturally out-crossed population was observed when *C. lineatus* was grown in the field. The plant had more of sterile grains than fertile grains (Saxena et al., 2010). When the plant was crossed with ICPL 99044 the progeny plants were partially male sterile. BC_4 population was grown in the field and a detailed study of the pollen grains was carried out. None of the sterile plants produced pods upon self pollination. Microscopic study of the pollen grains revealed that plants which did not produce pods with self pollination, stained red with aceto carmine. They were structurally fertile but functionally sterile. None of the grains germinated on pollen germination medium (Mallikarjuna and Saxena, unpublished). This system needs to be investigated further.

A_7 CMS system with *C. platycarpus* cytoplasm**:** Hormone aided pollinations coupled with embryo rescue techniques gave rise to F_1 and BC_1 generations in the cross *C. platycarpus* x *C. cajan*, which were already reported in different publications. BC_1 plants produced mature seeds. A selection was made in BC_2 population for male sterility. All the plants in this line called BC_2F_1-E had low pollen fertility. Pollen fertility in the lines ranged from 15 to 48 %. Crosses between BC_2F_1-E and ICPL 85010 gave rise to two plants with total pollen sterility namely BC_3-E15 and BC_3-E4. These two plants were crossed with cultivars Asha, ICPL 85010, ICP 1444, UPAS 120 and *C. platycarpus* accession ICPW 68.

Clasmogamous flowers were observed for the first time in BC_2-E plants and the trait persisted in BC_3 plants. Crosses between BC_3-E15 and cultivar ICPL 85010 gave rise to 7 % pod set. Pollen fertility in the progeny plants ranged from 0 to 3 %. Most of the anthers were sepalous, transparent and devoid of a regular anther cavity and pollen grains. Some of the anthers had a miniature anther cavity with a few pollen grains. In some of the anthers the pollen grains appeared to be fertile,

staining pink in aceto carmine. Staining them with Alexander's stain showed that some of the anthers with pink fertile grains when stained with aceto carmine, stained green. Dehiscence of the anthers did not take place in any of the anthers to release the pollen grains. Upon closer study it was observed that anther cavities lacked the line of dehiscence and had thick anther wall, which prevented the dehiscence of anthers and release of pollen grains. In fact, at the stage of pollen grain maturity, anther cavity developed multi-layers and a thickened cell wall. Since dehiscence did not take place, pollen grains were not released from the anther sacs. Anther sacs were forcefully ruptured by squashing the anthers and self pollinations were carried out. In spite of self pollinations none of the plants set seeds from self pollinations.

Meiotic study of the progeny revealed that metaphase proceeded normally with the formation of either seven bivalents and two tetravalents or with 11 bivalents. Anaphase proceeded to metaphase with normal disjunction of chromosomes resulting in tetrad formation. It was only after the formation of tetrads that many of the pollen grains lost their contents and turned sterile.

Crosses between BC_3-E15 and ICPL 87119 gave rise to an average of 21 % pod set. Pollen fertility in the plants ranged from 19 to 80 %. It was possible to get pods from self pollinations. Crosses between BC_3-E15 and ICP 88014 gave rise to 19 % pod set. The F_1 hybrids were fertile and pollen fertility in these plants ranged from 0 to 79 %, and only one plant had 100 % sterile pollen grains. Crosses with ICP 1444 gave rise to only fertile plants and pollen fertility in the plants ranged from 20 to 77 % (Table 1). Crosses with *C. platycarpus* ICPW 68 gave rise to 1.0 % pod set. Pollen fertility in the F_1 hybrids ranged from 15 to 38 %. Except for the progeny from the cross with ICPL 85010 whose progeny had high sterility, majority of the progeny obtained utilizing other cultivars had high fertility.

C. platycarpus is the only wild relative from the tertiary gene pool of pigeonpea which has been successfully crossed with the cultigen. Screening advanced generation progeny lines for *Phytophthora* blight (PB) and *Helicoverpa armigera* (HA) has helped to identify lines with low damage due to PB and low HA damage (Mallikarjuna et al., 2011a). More recently a few lines with resistance to *Fusarium* wilt (Patancheru isolate) and sterility mosaic (Patancheru isolate) disease has been identified (Mallikarjuna and Saxena, unpublished data). Identification of CMS from *C. platycarpus* cytoplasm (Mallikarjuna et al., 2011a) is another source for the exploitation of heterosis and diversification of the cytoplasmic base. It is envisaged that this system when fully developed should be able to effectively exploit heterosis, as *C. platycarpus* is a tertiary gene pool species and its cytoplasm may be very diverse from that of cultivated pigeonpea.

More recently, traits for CMS were observed in the progeny lines derived from *C. lanceolatus*. Efforts are underway to identify restorers and maintainers. Once confirmed this is a new sources derived from *C. lanceolatus* cytoplasm and will be named as A_8 CMS system.

References

Ariyanayagam, R. P., A. N. Rao, and P. P. Zaveri, 1993: Gene-cytoplasmic male-sterility in pigeonpea. *Int. Pigeonpea Newsl*., **18**: 7-11.

Ariyanayagam, R. P., A. N. Rao, and P. P. Zaveri, 1995: Cytoplasmic male-sterility in interspecific matings of *Cajanus*. *Crop Sci,*. **35**: 981-985.

Bond D.A., J.L. Fyfe, T.C. Clarke, 1966. male sterility in field beans IV. Use of cytoplasmic male sterility in the production of F_1 hybrids and their performance in trials. *J. Agric. Sci*., **66**: 369-377.

Howard, A., G.C. Howard and A.R. Khan, 1919. Studies in pollination of Indian crops. *I. Memoirs, Department of Indian Crops, India. Botanical Series*, **10**: 195-200.

Malikarjuna, N., and N. Kalpana, 2004: Mechanism of cytoplasmic nuclear male-sterility in pigeonpea wide cross *Cajanus cajan* x *C. acutifolius*. *Indian J. Genet.*, **64**: 115-117.

Mallikarjuna, N., and K. B. Saxena, 2005: A new cytoplasmic nuclear male-sterility system derived from cultivated pigeonpea cytoplasm. *Euphytica*, **142**: 143-148.

Mallikarjuna, N., and J. P. Moss,1995: Production of hybrid between *Cajanus platycarpus* and *Cajanus cajan*. *Euphytica*, **83**: 43-46.

Mallikarjuna, N., D. Jadhav, and P. Reddy, 2006: Introgression of *Cajanus platycarpus* genome into cultivated pigeonpea genome. *Euphytica*, **149**: 161-167.

Mallikarjuna N., K. B. Saxena and D. R. Jadhav, 2011a. *Cajanus*. In: Wild Crop Relatives: Genomic and Breeding Resources (C kole ed), pages 21-33. Springer Berlin Heidelberg, DOI: 10.1007/978-3-642-14387-8_2.

Mallikarjuna N., K B Saxena, Jhansi lakshmi, R Varshney, S Senthilvel, S Sandhya rani and D R Jadhav, 2011b. Differences between *Cajanus cajan* (L.) Millspaugh and *C. cajanifolius* (Haines) van der Maesen, the progenitor species of pigeonpea. Genet Resour Crop Evol (DOI: 10.1007/s10722-011-9691-8).

Saxena, K. B., R. V. Kumar, N. Srivastava, and S. Bao, 2005: A cytoplasmic-nuclear male-sterility system derived from a cross between *Cajanus cajanifolius* and *Cajanus cajan*. *Euphytica*, **145**: 289—294.

Saxena, K. B., and R. V. Kumar, 2003: Development of a cytoplasmic nuclear male-sterility system in pigeonpea using C.scarabaeoides (L.) Thouars. *Indian J. Genet,*. **63**: 225-229.

Saxena, K.B., Sultana, R., Mallikarjuna, N., Saxena, R.K., Kumar, R.V., Sawargaonkar, S.L. and Varshney, R.K. 2010. Male-sterility systems in

pigeonpea and their role in enhancing yield. *Plant Breed.*, **129**: 125-134.

Tikka, S.B.S., L.D. Parmar and R.M. Chauhan, 1997. First record of cytoplasmic-genic male-sterility system in pigeonpea (*Cajanus cajan* (L.) Millsp.) through wide hybridization. *GAU Res. J.*, **22**(2): 160–162.

.

Wanjari, K. B., A. N. Patil, P. Manapure, J. G. Manjaya, and P. Manish, 1999: Cytoplasmic male-sterility in pigeonpea with cytoplasm from *Cajanus volubilis*. *Ann. Plant Physiol*. ,**13**: 170-174.