Euphytica (2010) 173:397–407
DOI 10.1007/s10681-010-0140-0

REVIEW

An overview of male-sterility systems in pigeonpea [*Cajanus cajan* (L.) Millsp.]

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Received: 10 September 2009 / Accepted: 22 January 2010 / Published online: 11 February 2010

Abstract For commercial development of hybrids the four pre-requisites are; availability of perfect male-sterility system, efficient mass pollen transfer mechanism, hybrid vigor, and the large scale seed production of hybrids for commercialization. The type of male-sterility governs the acceptance of hybrids by farmers. Genetic male-sterility (GMS) system was not accepted by farmers due to the economics of large scale seed production. The major drawback was rouging of fertile counterpart from the female plot, which was time consuming and labor intensive. Cytoplasmic-genic male-sterility (CMS) system usually was a better option for large scale seed production. Hybrid vigor has been utilized in some cereal and vegetable crops. Pigeonpea (*Cajanus cajan*) displays considerable natural out-crossing and now CMS lines are available with different cytoplasmic backgrounds. This mini-review reports the research on development of CMS lines and CMS-based hybrids in pigeonpea.

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Keywords *Cajanus cajan* · Male-sterility systems · Pigeonpea

Introduction

Pigeonpea (*Cajanus cajan* (L.) Millsp.) is one of the most important legume crops of tropics and subtropics of Asia and Africa. Pigeonpea is a short-lived perennial shrub (Maesen et al. 1980), which plays a major role in improving soil nutrition by nourishing soil with atmospheric nitrogen fixation. The stems are used as domestic fuel wood and for making huts; leaves are used as fodder, while seeds are a primary source of protein for the poor vegetarian population (Saxena et al. 2006). Globally pigeonpea is grown on 4.67 million hectare area with production of 3.30 million tonnes (Table 1, www.fao.org). Asia contributes about 89% in global area and 87% in global production. India is the major pigeonpea growing country with 3.56 million hectare area (76% of global area) and 2.31 million tonnes production (70% of global production) (www.faostat.org). Pigeonpea is different from other legumes as it exhibited large variation (20–70%) in natural out crossing, so pigeonpea can be considered an often cross-pollinated crop (Saxena et al. 1990a). However, this considerable amount of natural out-crossing has been a problem in maintaining genetic purity of varieties,

Table 1 Global pigeonpea (FAOSTAT 2009[a])

Region/ country	Area (Million ha)	Production (Million tonnes)	Yield (kg ha^{-1})
World	4.67	3.300	707
Asia	4.15	2.870	692
India	3.56	2.310	649
Nepal	0.02	0.019	917
Myanmar	0.57	0.540	947
Bangladesh	0.06	0.015	961
America	0.04	0.029	795
Africa	0.48	0.400	829

[a] The data is available for the year 2007. www.faostats.org

but it has been used efficiently in hybrid breeding technology. As hand emasculation and pollination is commercially not feasible, development of male-sterile lines was considered the best way to utilize the available natural out-crossing in pigeonpea. For crops where seed is the economic component, either GMS or CMS systems can be used. Research for development of GMS lines started in 1970's and after a decade the first GMS-based hybrid in pigeonpea, ICPH 8 was released. The yield potential of the GMS-based hybrids encouraged scientists to develop CMS lines. The first stable CMS line with cytoplasm from a wild species *C cajanifolius* was reported by Saxena et al. (2005a). Efforts are being made at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India, for diversification of cytoplasmic and nuclear background of the CMS lines. This review summarizes development of male-sterility systems and their utilization in breeding high-yielding hybrids.

Genetic male-sterility systems

Inability of a normal plant to produce viable pollen grains is known as male-sterility. When this mechanism is governed by nuclear gene(s), it is designated as genic male-sterility. In genic male-sterility, the anomaly usually is monogenic recessive inheritance. These male-sterile plants exhibit delayed senescence and non-dehiscent, flat, translucent anthers with a whitish scaly surface. Male meiosis proceeds normally up to tetrad formation, after which degeneration of pollen mother cells (PMCs) occurs quickly in non-separated tetrad cells leaving behind an intact tapetum. In fertile plants, the tapetum degenerates during tetrad formation and microspore release. Another cause of male-sterility is early breakdown of tapetum layer, which provides nutrition to the developing PMCs (Kaul 1988).

Occurrence and perpetuation

Spontaneous

In about 175 species in which male-sterility has arisen spontaneously, the majority are dicots and some are from species crosses (Kaul 1988). In case of pigeonpea, first report of sterility was by Deshmukh (1959) in which, spontaneously arisen gametic sterility affecting both sexes occurred, but its genetic control was not known. Reddy et al. (1978) reports genic male-sterility systems in pigeonpea. They found six male-sterile plants, which did not produce pollen grains from two apparently unrelated sources in pigeonpea. Crossing success was normal indicating female fertility. All flower parts, except anthers, were normal in size and color in the male-sterile plants. Their anthers were smaller than normal anthers, flat, and translucent with whitish scaly surfaces. In crossing studies, all F_1s were fertile suggesting dominance of fertility. In the F_2 generation, all other combinations fit 3:1 ratio except for one cross. Therefore it was concluded that this male-sterility was controlled by a single recessive gene, for which gene symbol ms_1 was proposed. Wallis et al. (1980) reported a new source of genic male-sterility; abnormal anther development was found, which involved degeneration of the PMCs at the young tetrad stage. Dundas et al. (1981) studied microsporogenesis and anther wall development of male-sterile line developed from breeding line B15B. In sterile plants, PMC degeneration occurred at the young tetrad stage with the rupturing of each nuclear membrane and collapse of the outer cell wall. In the fertile plants, microsporogenesis proceeded quickly from PMC to mature bi-nucleate pollen grains.

Dundas et al. (1982) identified a male-sterile mutant plant in an elite photoperiod insensitive breeding line (QPL-2) of Indian origin. This was the third source of male-sterility in pigeonpea, the anthers of which were similar in appearance to those described by Wallis et al. (1980). Older anthers

nearing meiosis showed approximately twice the number of PMC's in male-sterile plants as in those of the male-fertile plants. First sign of PMC degeneration was the arresting of meiotic division at pachytene, followed by the thinning of the cytoplasm and its separation from the wall of each PMC. Anther wall development was delayed as compared with normal plants. Only three layers of cell wall were present even though young PMC's had formed. Restriction in the supply of nutrients to PMC's caused by under development of the tapetum was the probable cause of PMC degeneration in the sterile plants. Saxena et al. (1983) studied the inheritance of the B15B male-sterile character and its allelic relationship to the translucent anther type. All F_1 progeny of crosses of the three cultivars to male-sterile B15B plants were fertile and F_2 segregated for 3:1 (fertile:sterile) ratio. Further, the test cross progenies fitted 1:1 ratio. This strongly suggested the presence of a single recessive gene for male-sterility.

This was the beginning of the work on hybrid pigeonpea. Various studies were started to determine the performance of experimental hybrids and their genetic analysis. Omanga et al. (1992) studied grain yield in pigeonpea using male-sterile lines. Mean sum of squares for males, females, and their interaction were highly significant indicating importance of both additive as well as dominant gene action. MS Prabhat (DT), a determinate male-sterile line, was a good general combiner for dwarfness, earliness, and seeds per pod. Among the testers, C 11 was the best general combiner for yield.

Histological studies by Katti et al. (1994) showed differences between male-sterile and male-fertile pigeonpea lines. Pre-meiotic development was identical in anthers of both lines. During post-meiotic stages, male-sterile anthers showed persistent callose and tapetum. Breakdown of microspores occurred at late tetrad stage. It was postulated that the malfunctioning of the tapetum was the cause for the induction of male-sterility. Wanjari et al. (2000) reported a dominant genic male-sterility system derived from *Cajanus sericeus* × *Cajanus cajan*. Male-sterile plants of this cross segregated in BC_5F_2 generation and were maintained by sib mating. Due to dominant nature of male-sterile gene, in heterozygous condition, male-sterility was observed and hence it was considered as cytoplasmic-genic male-sterility.

Artificial production of male-sterility

Using wide hybridization

For production of hybrids, we should have good parental lines. Saxena et al. (1990b) identified potential parents for use in inter generic hybridization. *Cajanus scarabaeoides* (L.) Benth. had several useful genes, which were utilized for pigeonpea improvement. About 33 accessions were evaluated and a large amount of variation was observed. Only four accessions had more than 28% protein content. Wild accessions were resistant to *fusarium* wilt, *phytophthora* blight, sterility mosaic, and cyst nematodes. Accessions ICPW 89 and ICPW 111, in short duration and ICPW 94 and ICPW 118, in medium duration, were identified as potential parents for use in inter-generic hybridization. After identifying such parents, various male-sterile lines have been developed through wide hybridization and natural out crossing as *ms* Pusa 33, QMS 1, *ms* ICP 3783, and DAMS 1 (Pandey and Singh 1998).

Reddy et al. (2001) crossed *C. reticulatus* var. grandifolius a wild relative of the cultivated species, with *Cajanus cajan* as female parent and characterized the hybrid with fewer pods and seeds. The meiotic cells of the hybrid had reduced chromosome pairing, stickiness, and precocious movement of chromosomes to poles in the second meiotic division. Pollen fertility in the hybrid ranged from 26 to 58% while in Pant A2, it ranged from 93 to 97%.

Through chemicals

Streptomycin sulphate and sodium azide were used to induce male-sterility in pigeonpea varieties DA 11 and Pusa 9. S.K. Nagar (personal communication) has used Ethyl Methyl Sulphonate and Di Ethyl Sulphonate.

Through physical mutagen

Plants with varying degree of male-sterility/pollen sterility were isolated through induced physical mutagen as Gamma ray by scientists in India. After production of various genic male-sterile lines, efforts were made to produce hybrids in pigeonpea. After continuous efforts over a decade at ICRISAT,

scientists reported the world's first GMS based pigeonpea hybrid ICPH 8 (Saxena et al. 1993).

Constraints of genic male-sterility system

Though the hybrids developed with GMS-technology were high yielding as compared with the respective control cultivars, farmers did not accept these hybrids due to some constraints. The first was timely removal of 50% of the fertile counterpart from the seed production plot at the stage of flowering, which was not only costly but also time-consuming task. Additional cost incurred to the seed growers was the manual rouging of male-fertile plants. Inadequate knowledge of the seed growers regarding the multiple seed production systems was a concern. Quality of hybrid seed produced by farmers/seed growers was questionable. The profit margin for cereals was very attractive for private seed companies as compared to the legume pigeonpea.

Thus, it was proposed to find an early stage marker to identify the genic male-sterile plants, which would help to rouge the male-fertile plants from the female rows. This will be helpful for efficient utilization of the available resources.

Some promising GMS-based hybrids released in India

ICPH 8

ICRISAT released this hybrid in 1991 and it was the world's first GMS-based hybrid in pigeonpea (ICRISAT 1993). ms Prabhat (DT) and ICPL 161 were the parents of this hybrid. This hybrid had a semi-spreading and indeterminate growth habit. It flowered in 80–85 days after sowing. It took 115–135 days for 75% maturity. It showed wide adaptability and drought tolerance. However, it was susceptible to major pigeonpea diseases, but could withstand *fusarium* wilt and sterility mosaic diseases due to its earliness (ICRISAT 1991). This hybrid was released for cultivation in the central zone of India.

PPH 4

PPH 4 was another hybrid with Ms Prabhat (DT) as female parent and AL 688 as male parent. It matures within 150 days. In the All India Coordinated Trials, PPH 4 showed 32.1% yield superiority over the best national check variety UPAS 120 in the North Western Plains Zone (Verma and Sidhu 1993).

CoH 1

This hybrid was released in 1994 and ms T 21 was used as female parent, while ICPL 87109 was male parent. It was an indeterminate, short duration hybrid, which had an average height of 106 cm. This hybrid was mainly for cultivation in Tamil Nadu and Southern belts in India (Saxena et al. 2005a).

Cytoplasmic-genic male-sterility systems

Kolreuter (1763) was the first who observed cytoplasmic-nuclear male-sterility in intraspecific and interspecific hybrids. Correns (1908) reported that cytoplasmic factors could influence the occurrence of male-sterility and the trait would be maternally inherited.

Hybrid seed production technology in pigeonpea with genic male-sterility posses some problems, which involved prompt identification and removal of male-fertile sibs, which account for 50% of the population within female rows of a seed production plot. This job is time consuming and laborious, involving 40–45% of the seed production cost. Inefficiency in eliminating the sibs reduces the quality of hybrid seed (Verma and Sidhu 1993; Saxena et al. 1996). Due to these problems, searches for development of CMS systems was initiated in pigeonpea. In CMS system, there is interaction of nuclear and cytoplasmic factors. Specific genes of sterile cytoplasm interact with the fertility restorer gene of the nuclear genome to produce male-sterile phenotypes.

Wide hybridization—a method for CMS development

Ariyanayagam et al. (1995) reported development of a CMS system in pigeonpea by utilizing interspecific hybridization. They used ICPX 880227-10-1 as recurrent parent to cross with *Cajanus sericeus* accession EC 121208. Two approaches were followed to transfer nuclear genome of pigeonpea into

Cajanus sericeus cytoplasm. The first was wide hybridization involving conventional backcross method and other was multiple genome transfer. Large variations for male-sterility were observed at various genome transfer stages (GTS) and at GTS 4, 91–100% male-sterile plants were identified. ICPL 85030 and ICPL 90035 were promising maintainers. The preliminary cytological investigation of male meiosis in these CMS lines indicated that meiosis proceeded normally until the release of microspore, unlike the GMS systems. Soon after release, the vacuolation and degeneration of the protoplasm was observed. This cytoplasm source from *C. sericeus* was denoted as A_1 cytoplasm.

Tikka et al. (1997) made efforts for development of CMS lines using cytoplasm from *C. scarabaeoides*. The wild species accession was used as female parent. The F_1 plants were partially fertile with 2–3 small seeds per pod. The F_2 population was raised in isolation. Among the 95 F_2 plants, 14 plants were completely sterile. The completely male-sterile F_2 plants were crossed to four pigeonpea genotypes, QMS 2 (fertile), MS 288 (fertile), ICPL 87, and GT 100. The BC_1F_2 progenies of the crosses with MS 288 (fertile) and QMS 2 (fertile) exhibited 100% male-sterility. These results revealed the development of a new CMS system and the cytoplasm source was denoted as A_2 cytoplasm.

Rathnaswamy et al. (1999) studied crosses of wild relatives of pigeonpea with GMS lines. The GMS line *ms* 'Co 5' was crossed with two wild relatives of pigeonpea *C. cajanifolius* and *C. acutifolius*. All the F_1s of the cross *ms* 'Co 5' × *C. cajanifolius* were fully fertile whereas the F_1s of the cross *ms* 'Co 5' × *C. acutifolius* were partial sterile. The partial fertile F_1s were backcrossed to *ms* 'Co 5' and the fertile counterpart of 'Co 5' was crossed to the F_1s of *ms* 'Co 5' × *C. acutifolius*. In the F_1 generation of *ms* 'Co 5' × *C. acutifolius*, pollen sterility was 42.4–85.7%. This was a good effort for development of CMS, but 100% male-sterility was not observed.

Saxena and Kumar (2003) reported development of CMS line using *C. scarabaeoides* as female parent. ICPW 89, an Indian accession of *C. scarabaeoides*, was crossed with four pigeonpea lines ICPL 87051, ICPL 87119, ICPL 88039, and ICP 8863. The progeny of the cross ICPW 89 × ICPL 88039 was completely sterile. The anthers of the male-sterile plants were translucent and devoid of fertile pollen grains. The BC_2F_1 male-sterile plants were crossed with 14 pigeonpea lines in an attempt to understand the fertility restoration of this CMS system. Out of 14 crosses with CMS 88039A, six crosses had no pod set. In five crosses, the fertility restoration ranged between 94 and 100% and in the remaining three crosses the fertility restoration was partial. The results revealed the potential of this CMS system. Furthermore, this male-sterility trait can easily be transferred to other genotypes of different phonologies.

Chauhan et al. (2004) demonstrated the fertility restoration in A_2 CMS system. To identify perfect pollen fertility restorers, 543 derivative lines of F_5 and F_6 populations of *Cajanus scarabaeoides* × *Cajanus cajan* and 1365 germplasm accessions were used as pollen parent on A_2 CMS line GT 288A. The F_1s were evaluated for six seasons and, based on pooled data, eight promising fertility restorers were identified and characterized.

Saxena et al. (2005b) reported a stable male-sterile line through wide hybridization. ICPW 29, an accession of *C. cajanifolius* was crossed as female parent with a short-duration cultivar ICP 11501. Subsequently backcrosses were made to develop BC_6F_1 population. They reported that all the 1133 plants in BC_6F_1 generation were complete male-sterile. These results were confirmed again in BC_7F_1 generation. The CMS system with cytoplasm from *C. cajanifolius* was denoted as A_4 CMS system. Diversification of this male-sterile line is under progress at ICRISAT.

Mallikarjuna and Saxena (2005) reported a new CMS system, where cytoplasm donor was cultivated pigeonpea. This is the first report where male-sterility was reported using cytoplasm from cultivated pigeonpea. Six pigeonpea cultivars ICP 1140, ICPL 2, ICPL 85010, ICPL 85030, ICPL 88014, and ICPL 88034 were crossed with *C. acutifolius* accessions ICPW 15613 and ICPW 15605. The frequency of male-sterile and male-fertile plants in F_1 generation varied considerably from cross to cross. The cross combinations with *C. acutifolius* accession ICPW 15613, on average produced 18.3% male-sterile plants while it was 12.1% when crossed with accession ICPW 15605. The pollen sterility in the F_1s ranged from 40 to 100%. Unlike the previous reports, delayed degeneration of pollen mother cells was identified as the prime cause for the manifestation of this male-sterility system. This CMS system was denoted as A_5 CMS system.

Histological aspects of male-sterility

To know the cause for manifestation of male-sterility, microsporogenesis was studied. The degeneration of the tapetum by vacuolation was responsible for pollen mother cell breakdown in GMS line Dundas et al. (1981), whereas Dundas et al. (1982) reported earlier breakdown of microsporogenesis. Dundas et al. (1987) studied meiotic behavior of hybrids of pigeonpea and two Australian native *Atylosia* species. Meiosis in the parental pigeonpea and *Atylosia* accessions appeared regular, while that in the hybrids showed a lower frequency of ring bivalents at metaphase I. However, multivalent, univalent, rod bivalents, chromatin bridges, fragments, laggards, micronuclei, and supernary microspores occurred in meiotic cells of hybrids. Meiosis proceeded normally until the release of microspores. Soon after the release, vacuolation and degeneration of the protoplasm was seen. In contrast, in the genetic male-steriles, meiotic failure occurred at pollen mother cell or at earlier to late tetrad stages (Ariyanayagam et al. 1995). Reddy et al. (2001) studied the meiotic behavior of hybrids between *Cajanus cajan* × *Cajanus reticulatus* var. *grandifolius*. The meiotic cells of the hybrid had quadrivalents, trivalents, univalents, and showed reduced chromosome pairing as revealed by the increased number of rod bivalents per cell at metaphase I. In addition, stickiness and precocious movement of chromosome to poles in the second division was observed. Mallikarjuna and Saxena (2002) found 96% normal chromosome segregation at metaphase with 11 bivalents, confirming the findings of Dundas et al. (1987).

Jogendra et al. (2004) studied cytogenetic analysis of interspecific hybrids in genus *Cajanus*. Some meiotic abnormalities were observed in the F_1s of ICPL 84023 × *Cajanus acutifolius*, UPAS 120 × *Cajanus acutifolius*, and PA 134 × *Cajanus acutifolius*, indicating varying degrees of chromosome and genic differences between *Cajanus cajan* and *Cajanus acutifolius*. These observations again confirmed that *Cajanus cajanifolius* was the closest wild relative of *Cajanus cajan*, followed by *Cajanus scarabaeoides*, *C. sericeus*, and *C. acutifolius*. Mallikarjuna and Kalpana (2004) studied the mechanism of cytoplasmic-nuclear male-sterility in pigeonpea derived from cross of *Cajanus cajan* × *Cajanus acutifolius*. They found two types of mechanisms. Type I CMS had partially or totally brown and shriveled anther morphology and the process of microsporogenesis was inhibited at the pre-meiotic stage. Type II CMS plants had pale white shriveled anthers and the breakdown in microsporogenesis was at the post meiotic stage after the formation of tetrads. Dalvi et al. (2008a) studied the micropsorogenesis in CMS line of pigeonpea with A_4 cytoplasm. They defined that the breakdown of tapetum is the main cause for male-sterility (Fig. 1).

From all the reports, it is clear that, the major reason of sterile F_1s of interspecific crosses lies in the abnormal pairing of chromosomes. In most of the cases ring bivalents were formed, which was result of a chromosome inversion. Therefore, we can predict that inversion is one of the reasons, which leads to production of male-sterile progeny in interspecific hybridization.

Genetics and fertility restoration studies

Dalvi et al. (2008a) studied the inheritance of fertility restoration. For inheritance study, male-sterile line ICPA 2039 was crossed with five inbred restorers ICP 12320, ICP 11376, HPL 24-63, ICP 10934, and ICP 13991. The F_1s of all the crosses were grown at ICRISAT, Patancheru during 2005 rainy season. The individual F_1 plants were selfed with muslin cloth bags to produce F_2 seeds and the F_1 plants were backcrossed to the female parent to produce BC_1F_1 generation seeds. The parents, F_1, F_2, and BC_1F_1 populations were grown during 2006 rainy season at Patancheru. Data on segregation for male-sterility and fertility were recorded in each generation and standard χ^2 method was applied to test the goodness of fit for various segregation ratios. All the F_1 plants in five crosses were fully fertile indicating the dominance of fertility restoring gene(s). The F_2 and BC_1F_1 progenies of the crosses involving A_4 CMS line segregated for male-sterility and fertility (Table 2). Among the five crosses studied, three (ICPA 2039 × ICP 12320, ICPA 2039 × ICP 11376, and ICPA 2039 × HPL 24-63) segregated in a ratio of 3 fertile:1 sterile in F_2 generation, whereas in the backcross generation a segregation of 1 fertile:1 sterile was observed. This indicated monogenic dominance nature of fertility restoring gene. The pooled values over the three crosses also exhibited goodness of fit for 3 fertile:1 sterile ($\chi^2 = 0.11$) ratio in F_2 generation and 1 fertile:1

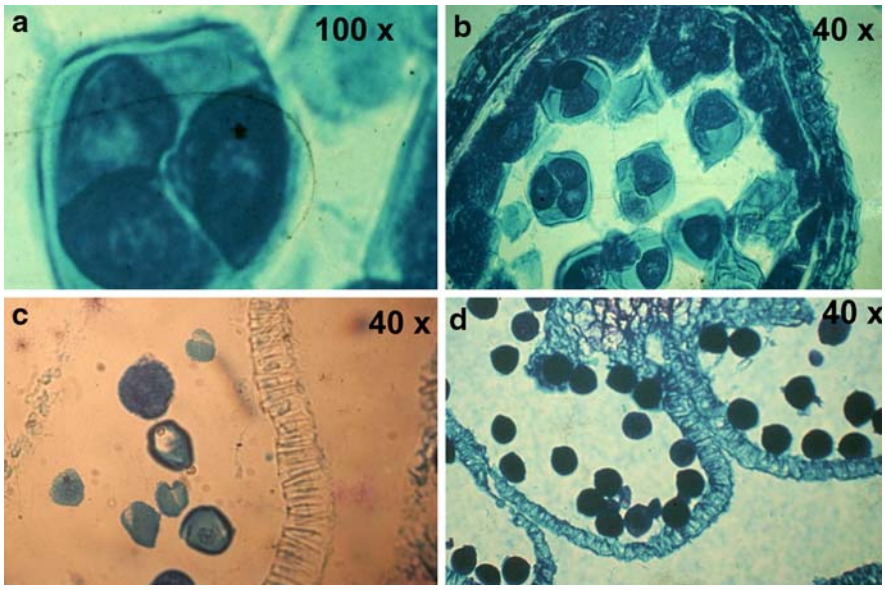

Fig. 1 (**a**–**d**) Transverse section of anthers of male-sterile (*left*) and male-fertile (*right*) plants of pigeonpea from tetrad stage to pollen grain development stage. **a** and **b** Tetrad stage pollen of male-sterile and male-fertile plants, respectively; **c** and **d** mature anthers of male-sterile (*undeveloped*) and male-fertile plants (*completely developed*), respectively

sterile ($\chi^2 = 1.06$) in backcross generation. The F_2 and backcross populations of cross ICPA 2039 × ICP 10650 segregated in the ratio of 15 fertile:1 sterile ($\chi^2 = 0.31$) and 3 fertile:1 sterile ($\chi^2 = 0.44$), respectively. This suggested the involvement of two dominant genes with duplicate gene action. The other cross (ICPA 2039 × ICP 13991) segregated in a ratio of 9 fertile:7 sterile ($\chi^2 = 2.10$) in F_2 generation and 1 fertile:3 sterile ($\chi^2 = 3.86$) in BC_1F_1 generation indicating the presence of two complimentary genes for restoring the fertility of male-sterile line. In pearl millet 1–3 dominant genes (Yadav 2005) and in *Vicia faba* 1–2 dominant genes (Kaul 1988) governed the fertility restoration. Further studies are needed with isogenic lines for confirmation of these results.

Some of the germplasm from ICRISAT, Patancheru, and Marathwada Agricultural University, Parbhani was used for fertility restoration studies. The newly developed cross combinations were planted as un-replicated three-row plot trial at three locations viz. Patancheru (Andhra Pradesh), Parbhani (Maharashtra), and Latur (Maharashtra). Some of the male parents like ICPA 129-3, Nirmal 2, BWR 23 were good fertility restorers for A_1 cytoplasm CMS line ICPA 2067 and can be used for development of hybrid combinations. ICPA 129-3, Nirmal 2, and BSMR 175 were male-sterility maintainers of A_2 cytoplasm CMS line ICPA 2052. In case of ICPA 2039, one perfect male-sterility maintainer (ICPA 129-3) and fertility restorer (BSMR 736) were identified. Some of the lines were segregating for fertility restoration and plant-to-plant crosses will be better for accurate identification of male-sterility maintainers and fertility restorers.

Natural out crossing and further studies

For the efficiency of any commercial hybrid seed production, we need an effective natural mass pollen transfer mechanism. The first report of natural out crossing in pigeonpea was in 1919 (Howard et al. 1919). They reported 14% natural out crossing in pigeonpea. Saxena et al. (1990a) reported wide range of natural out crossing (0–70%) at various locations in India. After development of cytoplasmic-genic male-sterile lines, however, there was a need to diversify the present male-sterile lines. Efforts were made to identify some lines of wild species as parents for inter-generic hybridization to identify some lines as tester for the production of hybrids. Saxena et al. (1990b) studied variation in 33 accessions of *Atylosia scarabaeoides* (L.) Benth. They found that *A. scarabaeoides* accessions were less susceptible to *Lepidopteron* borer and were immune to pod fly damage. Two short and two medium duration

Table 2 Segregation for male-sterility and fertility in F_1, F_2, and BC_1F_1 populations of crosses involving CMS line ICPA 2039 and five fertility restorers in pigeonpea

Cross	Number of plants													
	F_1 generation			F_2 generation						BC_1F_1 generation				
	Total	Fertile	Sterile	Total	Fertile	Sterile	χ^2 (expected)	χ^2 (calculated)	P (0.01)	Total	Fertile	Sterile	χ^2 (expected)	χ^2 (calculated)	P (0.01)
ICPA 2039 × ICP 12320	27	27	0	428	317	111	3:01	0.20	0.66	103	42	61	1:01	3.50	0.06
ICPA 2039 × ICP 11376	30	30	0	430	312	118	3:01	1.37	0.24	158	68	90	1:01	3.06	0.08
ICPA 2039 × HPL 24-63	25	25	0	471	373	98	3:01	4.42	0.04	115	68	47	1:01	3.83	0.05
Pooled	82	82	0	1329	1002	327	3:01	0.11	0.74	376	178	198	1:01	1.06	0.30
ICPA 2039 × ICP 10650	32	32	0	179	166	13	15:01	0.31	0.58	108	78	30	3:01	0.44	0.50
ICPA 2039 × ICP 13991	30	30	0	518	275	243	9:07	2.1	0.15	112	37	75	1:03	3.86	0.04

Source: Dalvi et al. 2008a

accessions were identified as parents for use in intergeneric hybridization.

Nageshwar Rao et al. (1996) studied difference between natural and hand pollination. They observed 62.5% success in hand pollination. Maximum of 79 pods per plant were observed under natural pollination. This pollination studies suggested that CMS plants were capable of an acceptable level of pod set under natural pollination. In addition, these studies were helpful for identification of efficient pollen parent. Chauhan et al. (2004) studied fertility restoration in cytoplasmic-genic male-sterile line of pigeonpea derived from *Cajanus scarabaeoides*. They tested 1908 accessions on GT 288A. From this study, 18 fertility restorers were identified and characterized. Mallikarjuna and Saxena (2005) reported a new cytoplasmic-nuclear male-sterility system derived from cultivated pigeonpea cytoplasm. *Cajanus acutifolius* was one of the wild relatives of pigeonpea used as pollen donor. This is the first report of development of CMS with cultivated pigeonpea cytoplasm. Several restorers for this CMS system were identified but maintainers from cultivated type was the problem in this system. The fertility restoration and male-sterility maintenance systems operating within the hybrid progenies were incomplete and may be due to the presence of some major differential intergenomic or cytoplasmic-genomic interactions. Saxena et al. (2005b) reported a stable male-sterility system derived from *C. cajanifolius* cytoplasm designated as A_4 cytoplasm. The experimental hybrid developed from this A_4 CMS system, ICPH 2470 exhibited 77.5% yield advantage over control cultivar UPAS 120.

Pod setting with natural out-crossing

In any seed production program, proper isolation distance is needed for pure seed production of parents and hybrids. In pigeonpea, the natural mass transfer of pollen grains was accompanied by a number of pollinating insects (Onim, 1981). Howard et al. (1919) reported 14% natural out-crossing at Delhi, India whereas in a review by Saxena et al., (1990a) a range (0–70%) of natural out-crossing was reported from various locations in India.

The nucleus seed of parental lines of hybrids should be produced with highest standards of genetic purity. For multiplying nucleus seed, both A- (male-sterile line) and B- (male fertile counterpart of

A-line) lines should be grown, preferably inside an insect proof cage. Individual plants of A- and B-lines should be examined for various parameters and off-types be rouged and crosses should be done to produce the desired number of seeds. For the production of breeder's seed of A-line, a field with appropriate isolation distance is selected and A- and B-lines are grown with recommended agronomic package. At ICRISAT, a ratio of 4 rows of A-line: 1 row of B-line was effective in producing seed of a given A-line. In the seed multiplication of ICPA 2039 at ICRISAT, 27 kg of crossed seed was harvested from 225 m^2 block with an estimated yield of 1111 kg ha^{-1}. In another isolation of the same male-sterile line, 200 kg seed @ 877 kg ha^{-1} was harvested (Saxena 2006a). ICPA 2043—a medium-duration A-line yielded 1270 kg seed ha^{-1}. The amount of seed yield obtained with natural out-crossing is encouraging for commercialization of hybrids in pigeonpea.

Seed production of hybrids

In a three-line hybrid seed production system, the hybrid seed produced by crossing A- line with R-line, is commonly called certified seed and it is grown on a larger scale. For the production of certified seed of hybrids the A-line and its pollen parent (R-line) are grown in 4:1 ratio in an isolated block. Some additional rows of pollen parent can also be sown on each side of the plot. This will enhance pollen availability for cross pollination. At flowering the pollinating insects will visit the male and female flowers in a random fashion and in the process collect pollen from fertile plants and carry out hybridization on the male-sterile plants. Using this technology in 2005 rainy season, from a small (120 m^2) isolation a total of 15 kg seed @ 1250 kg ha^{-1} of hybrid ICPH 2671 was produced (Saxena 2006b). For optimum seed yields the male:female row ratio (1:4) should not be recommended for all environments, but depends on insect activity and time of sowing.

Transfer of male-sterility to productive backgrounds

At present, male-sterile lines are available in short-, medium-, and late-duration in A_4 cytoplasmic background. The experiences in other crops such as maize revealed the danger of utilizing a single cytoplasmic source. Therefore, it is necessary to diversify the male-sterility systems in pigeonpea. Though four male-sterility systems are available (Saxena et al. 2006), only A_4 CMS system is being utilized now by ICRISAT and its public–private partners. For diversification of male-sterility system in productive background, the basic need is to have locally adapted genotypes with biotic and abiotic stress resistance. Dalvi et al. (2008b) made efforts to identify the fertility restoration of different CMS lines with the same set of male-parents. To transfer the male-sterility trait, large number of crosses have been made at ICRISAT. The cross combinations were tested in field for male-sterility trait. Some of the crosses exhibited >90% male-sterility and subsequent backcrosses were made on the sterile plants with respective recurrent parents for transfer of nuclear genome. Some of the crosses were >90% fertile, which were evaluated for hybrid vigor. Few cross combinations were segregating for male-sterility trait and these crosses were repeated by single-plant crossing program.

Identification of phenotypic markers

For grow out test (GOT), it is necessary to have a quick assessment system to identify pure hybrid seed. As pigeonpea is grown as annual crop, days to flowering takes a long time and it is very difficult to conduct a quick GOT. Therefore, some phenotypic markers, which help in easy and efficient identification of pure hybrid seed within short time period are needed. At ICRISAT, two leaf markers have been identified (obtuse and ovate leaf), which are governed by single recessive genes. Incorporation of these leaf markers into CMS background will be helpful for easy identification of pollen shedders in seed production program.

Summary

Stable cytoplasmic-genic male-sterility systems have been developed, which will be helpful for economic hybrid pigeonpea. However, there is need to widen the genetic base of CMS lines because, now only primary and secondary gene pools have been utilized. We need some help of molecular tools for efficient utilization of tertiary gene pool. This will be helpful for the production of hybrids for greater stability for

drought tolerance and disease resistance. The exploitation of heterosis and restructuring of plant type are two possible ways of increasing yielding ability of pigeonpea. In pigeonpea, the first break through in yield is likely to come from hybrids and the second by modifying the plant type (Saxena et al. 2005a). At present, all the important biological systems necessary for a successful hybrid-breeding program are available in pigeonpea and these need to be utilized efficiently for commercialization.

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