Review

Male-sterility systems in pigeonpea and their role in enhancing yield

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Abstract

Male-sterility has been successfully used for enhancing yield in a number of cereal and vegetable crops. In food legumes, this technology could never be used either due to non-availability of natural out-crossing system, or an efficient male-sterility system or both. Pigeonpea [*Cajanus cajan* (L.) Millsp.] is a partially cross-pollinated food legume and recent success in breeding a stable male-sterility system has allowed breeders to exploit hybrid vigour for increasing yields. The cytoplasmic-nuclear male-sterility (CMS)-based hybrids have recorded 28.4% yield superiority over local checks in farmers' fields. This paper besides summarizing the reports of all the genetic and CMS systems, also discusses the prospects of utilizing these male-sterility systems in commercial hybrid breeding programmes.

Key words: *Cajanus cajan* (L.) Millsp. — pigeonpea — genetic male-sterility — cytoplasmic-nuclear male-sterility — micro-sporogenesis — natural out-crossing — hybrid vigour

Male-sterility in plants is a phenomenon where the individuals are unable to reproduce through natural means because of their defective male-reproductive parts. Such plants reproduce only when fertile pollen from other plants is placed on the stigmatic surface of the male-sterile flowers through any mechanical means such as deliberate manual efforts, wind or insects. The phenomenon of male-sterility was recorded as early as by Kolreuter 1763. Subsequently, its role in evolution particularly that of dioecism was proposed by Darwin (1890). Bateson et al. (1908) suggested that male-sterility in most cases was controlled by recessive genes. Correns (1908) was the first to demonstrate the role of some cytoplasmic (maternal) factors in the expression of male-sterility. Subsequently, a large number of reports have appeared on various aspects of male-sterility in different crops. Such events can be brought about either by nature or through human interventions. Like other traits in living organisms, the male-sterility is also governed by specific genetic factors which are generally recessive in nature. Such genes are exposed during inbreeding and their mainte-nance is affected by fertilization with the pollen that carries counterpart dominant gene(s).

Male-sterility systems have played a great role in enhancing productivity of many crops through exploitation of hybrid vigour. In a plant system, the male-sterility is generally caused by some specific bio-chemical events that hinder the normal biological processes of pollen production. It is also observed that all the male-sterility systems identified so far in different crops could not be used in hybrid breeding programmes because of non-availability of other complementary genetic systems required for restoring their male-fertility. For effective utiliza-tion of a male-sterility system in hybrid breeding, it is important that the expression of both the male-sterility and its fertility restoration are stable over years and locations. Such male-sterility systems have been successfully exploited in breeding high-yielding hybrid cultivars in sorghum [*Sorghum vulgare* (L.) Monach], pearl millet [*Pennisetum glaucum* (L.) R. Br.], maize [*Zea mays* (L.) spp. mays], sunflower [*Helianthus annus* (L.)], castor [*Ricinus communis* (L.)] and more recently in rice [*Oryza sativa* (L.)] and wheat [*Triticum aestivum* (L.)]. Besides these, in a number of fruits and vegetable crops also, breeders have successfully exploited hybrid vigour for enhancing their pro-ductivity. Food legumes, on the contrary, could not take advantage of this phenomenon because of their highly self-pollinating nature that restricts large-scale seed production of hybrids. Therefore, raising the productivity of food legumes has been a long standing challenge for breeders.

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is an important high protein (20–22%) food legume of rainfed tropics and sub-tropics. Globally, it is cultivated by small holding farmers on 4.9-m ha in Asia, Africa and South America. It is primarily consumed as decorticated dry splits, fresh or frozen peas. In spite of high importance in nutrition of poor masses and dedicated efforts of scientists, the productivity of pigeonpea in the last five decades has remained low at about 700 kg/ha (http://faostat.fao.org/site/339/default.aspx). Pigeonpea is unique among legumes as its floral morphology allows both self – as well as insect-aided cross-pollination and their extents vary from one place to another. However, most breeders in the past ignored this fact and handled pigeonpea as a self-pollinated crop as far as its breeding methodology was concerned. The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), located at Patancheru (India), focused pigeonpea research on hybrid breeding by exploiting its partial (25–70%) natural out-crossing (Saxena et al. 1990). To achieve success in this endeavour, it was essential to breed a quality male-sterility system that would be acceptable to commercial hybrid seed producers. In the past 35 years, a considerable research was undertaken by ICRISAT on the breeding of various male-sterility systems in pigeonpea (Saxena 2009). This paper, besides reviewing the origin and nature of available male-sterility systems, discusses major achievements in breeding high-yielding hybrids.

Genetic Male-Sterility (GMS) Systems in Pigeonpea

All the GMS systems reported so far in pigeonpea have emerged from spontaneous mutations. This happens when a male-fertility controlling dominant (*Fr*) nuclear gene mutates to its recessive form under the influence of some natural forces and with subsequent natural selfing of heterozygotes (*Frfr*) the male-sterile genotypes (*frfr*) appear within the population. Such genotypes, if not cross-pollinated by fertile pollen, are eliminated from its parental population. In comparison with highly self-pollinated crops, the elimination of *frfr* genotypes is gradual in out-crossed species. Therefore, such elimination processes depend on the rate of natural out-crossing in a given population. In comparison with recessive genes, the frequency of dominant male-sterile genes in nature is very low (Kaul 1988). There are many instances where progeny of some inter-specific and -generic crosses have also produced male-sterile segregants. In genus *Cajanus* also, a number of such wide crosses have produced male-sterile segregants. These cases, however, were hardly pursued further (Dundas 1990, Reddy et al. 1990) for use in breeding programmes.

The male-sterile mutants have also been reported in some mutagen-induced populations. In most cases, such mutants could not be maintained either due to their tight association with female-sterility or reproductive abnormalities such as chromosome addition or deletion (Dundas 1990). If for some reason, a chromosome with male-fertility (*Fr*) gene is lost then male-sterility trait with *frfr* alleles will express but such plants hardly reach their maturity due to poor vigour and abnormal growth. In contrast, if the male-sterile mutant gene is dominant then it is eliminated rapidly from the population, particularly in a self-pollinated species. Therefore, most spontaneous male-sterile mutants that have been detected so far, are recessive. Relatively high occurrence of non-allelic recessive male-sterility genes suggests that the frequency of such natural mutations is quite high and their deletion from the parental populations is rather slow. According to Kaul (1988) the male-sterility in legumes that is controlled by recessive genes was reported in broad bean [*Vicia faba* (L.)], grass pea [*Lathyrus sativus* (L.)], groundnut [*Arachis hypogea* (L.)], sunhemp [*Crotalaria juncea* (L.)], soybean [*Glycine max* (L.) Merr.], pea [*Pisum sativum* (L.)] white clover [*Trifolium repens* (L.)], common bean [*Phaseolus vulgaris* (L.)], alfalfa [*Medicago sativa* (L.) spp. *sativa*] etc.; while dominant genetic control of male-sterility was reported in *Trifolium repens*.

Reports of genetic male-sterility systems in pigeonpea

Various GMS systems reported in pigeonpea are summarized in Table 1. The first report on male-sterility in pigeonpea was published by Deshmukh (1959). This spontaneous mutant could not be maintained because of its tight linkage with female-sterility. Reddy et al. (1977) made a deliberate search for male-sterility in 7216 germplasm accessions sown at ICRISAT in 1974. They selected 75 single plants which remained green till the end of season and had a few pods, suggesting absence of self-fertilization of flowers to affect normal pod setting. These selections were female fertile and had different types of anthers with variable fertility levels. Among these, six plants with fully grown translucent anthers and no pollen grains were selected for further studies and use in hybrid breeding programmes.

Dundas et al. (1982) reported a male-sterile mutant within a photo-insensitive pigeonpea breeding line. At about the same time yet another genetic male-sterile spontaneous mutant was selected in a breeding line B15B (Saxena et al. 1983). This mutant was characterized by brown coloured arrow-head shape anthers. Verulkar and Singh (1997) reported another recessive male-sterile mutant in a population of cultivar 'UPAS 120'. This mutant had translucent anthers, sparse podding and delayed flowering.

Wanjari et al. (2000) recorded the first dominant gene in an inter-specific progeny that controlled male-sterility in pigeonpea. Saxena and Kumar (2001) reported a genetic male-sterile mutant that was selected from an inbred population of cultivar 'ICPL 85010'. This mutant was characterized by small light yellow anthers with no pollen grains. Venkateswarlu et al. (1981) and Pandey et al. (1994) reported perhaps the similar male-sterile gene that was linked to characteristic obcordate leaves. In a segregating population of cross between obcordate leaf genotype and cultivar 'HY 3C', a total of 13 obcordate leaf type plants were found with 60–100% pollen sterility (Venkateswarlu et al. 1981). The authors postulated a linkage between male-sterility and obcordate leaf trait. They further observed that all the male-sterile plants had modified keel that exposed the flowers for out-crossing. Saxena et al. (1981) reported the presence of partial male-sterile plants with sparse pollen production in an F_2 population of cross MS 4A × QPL-1. The pollen sterility in these plants ranged from 40% to 80%. There was no intra-plant variation for pollen sterility. The pod set on these plants varied in accordance with their pollen-fertility. Gupta and Faris (1983) reported the identification of 11 male-sterile plants in a population of cross 0DT × ICPL 86.

Table 1: A summary of genetic male-sterility systems reported in pigeonpea

S. No.	Authors	Gene symbol	Remarks
1	Deshmukh (1959)	–	Male-sterility was associated with female-sterility
2	Reddy et al. (1977)	–	Seven types of floral variants with varying degree of male-sterility recorded
3	Reddy et al. (1978)	ms_1	Translucent male-sterile anthers
4	Dundas et al. (1982)	–	Photo-insensitive male-sterile mutant
5	Saxena et al. (1983)	ms_2	Brown, arrow-head shape anthers; non-allelic to ms_1
6	Verulkar and Singh (1997)	–	Single recessive gene control
7	Wanjari et al. (2000)	–	Single dominant gene control
8	Saxena and Kumar (2001)	ms_3	Under-developed anthers, non-allelic to ms_1 and ms_2
9	Venkateswarlu et al. (1981)	–	Male-sterility linked to obcordate leaf type
10	Pandey et al. (1994)	–	Male-sterility linked to obcordate leaf type
11	Saxena et al. (1981)	–	Partial male-sterility with sparse pollen production
12	Gupta and Faris (1983)	–	Recessive gene control

Anthers of the male-sterile plants were small, white (later turned brown) and non-dehiscent. They also reported another mutant with non-dehiscent type of male-sterility where the pollen grains were released only when the mature anthers were physically ruptured. The pollen thus obtained was 70–80% sterile. These mutants were not studied further.

Cytoplasmic-Nuclear Male-Sterility (CMS) Systems in Pigeonpea

The other type of major reproductive abnormality leading to male-sterility is caused together by specific nuclear and cytoplasmic genetic factors. In most cases, the recessive nuclear genes interact with specific genetic factors housed in the cytoplasm of a cell and make an individual's anthers non-functional leading to male-sterility. Such plants produce fertile pollen when the recessive nuclear genes are replaced by their dominant counterparts or the cytoplasmic male-sterility causing factors by fertility inducing genetic factors. Therefore, for maintaining a male-sterile progeny and to produce fertile hybrids, the individual pollen parents with different genetic constitutions are required. Hence, the hybrid breeding technology based on this system involves three parents; a male-sterile (A-) line, its maintainer (B-) line and a fertility restorer (R-) line.

The CMS systems can arise either through spontaneous mutation, intra-specific crosses, inter-specific crosses or inter-generic crosses. The wide hybridization programmes such as inter-specific and -generic crosses have been found to produce a greater proportion (about 75%) of CMS systems (Kaul 1988). Scanning of literature on this subject shows that in the dicots most CMS cases have arisen through inter-specific crosses, while in monocots it is the inter-generic hybrids that have yielded most CMS sources (Kaul 1988). As the expression of CMS requires two different genetic systems, one each in cytoplasm and nucleus, to come together in a single cell; the frequency of spontaneously occurring mutants simultaneously in both the entities (i.e., nucleus and cytoplasm) is quite low. On the contrary in GMS system, only a single nuclear mutation can lead to the development of male-sterility. Unlike GMS controlling genes, the influence of environment (temperature and/or photoperiod) on CMS controlling nuclear fr and Fr genes is more prominent. This may lead to instability of the expression of male-sterility and its fertility restoration. Such unstable expressions are also sometimes influenced by the genetic background of an individual.

Breeding of CMS lines in pigeonpea

Reddy and Faris (1981) made the first attempt to breed a CMS line in pigeonpea using cytoplasm of a wild relative of pigeonpea, then classified under a separate genus *Atylosia*. To start this programme, they crossed a cultivated type (as female) with pollen from two different wild relatives, *Atylosia sericea* and *Atylosia scarabaeoides*. The fertile F_1 plants of these two crosses were used as male parent to produce backcrosses with wild species as female parents. The resultant BC_1F_1 plants were male fertile while their BC_1F_2 progeny segregated for male-sterility and fertility. The maternally inherited male-sterility in these segregants was found to be tightly linked with various floral abnormalities such as petaloid anthers, free stamen or heterostyly. They also reported that these segregants had different degrees of

female-sterility and, could never be stabilized as pure lines therefore, could not be used in hybrid breeding programmes. Ariyanayagam et al. (1993) attempted to develop CMS through chemical and physical mutagens. A GMS line with ms_2 gene, when treated with 0.025% sodium azide or 500 mg/kg of streptomycin sulphate, showed mutational changes and expressed male-sterility that was maternally inherited. This male-sterility was maintained only by heterozygote sibs that raised doubts about its nature and use in hybrid breeding programme. The proportion of male-sterile plants in these mutagenic progeny varied a lot and no good male-sterile line could be derived Subsequently, a few CMS systems were developed in pigeonpea and these are briefly described below.

CMS with *Cajanus sericeus* (Benth.ex Bak.) van der Maesen comb. nov. (A_1) cytoplasm

Ariyanayagam et al. (1993) crossed *C. sericeus* with an advanced breeding line of pigeonpea. The F_1 progeny of this cross showed partial male-sterility but in F_2 generation a few segregants expressed 100% pollen sterility. In the subsequent backcross generations, for some reasons, these male-sterile plants could not maintain their high levels of male-sterility. In addition, it was also observed that some male-sterile plants reverted back to male fertility when local environment, particularly temperatures and photoperiods changed.

To stabilize the male-sterile trait, besides conventional backcrossing, Ariyanayagam et al. (1995) also used multiple cross genome transfer methodology. Both these approaches yielded certain proportion of male-sterile segregants, but the backcross derivatives were also found to be female-sterile and failed to set any pod. The progeny derived from the genome transfer scheme also produced a few male-sterile segregants which were maintained by other pigeonpea inbred lines. Saxena et al. (1996) carried forward the selections of Ariyanayagam et al. (1995) through additional hybridization and selection of male-sterile plants. This led to the development of male-sterile lines such as CMS 85010A, CMS 88034A and CMS 13091A (K. B. Saxena, Unpublished data). From these populations, Saxena et al. (2006) selected male-sterile lines that revert back to full male fertility under low temperature and shorter days (Table 2) and again to full male-sterility under high temperature and longer days.

CMS with *Cajanus scarabaeoides* (L.) Thou. var. pedunculatus (Reynolds and Pedley) van der Maesen comb. nov. (A_2) cytoplasm

In an attempt to develop a stable CMS line, Ariyanayagam et al. (1993) crossed *C. scarabaeoides* as female parent with a pigeonpea line ICPL 85030. The F_1 plants were partial male-sterile. In the backcross progeny, some promising male-sterile plants were identified but no stable CMS line could be bred. Tikka et al. (1997) reported the development of a CMS line by crossing a cultivated type with its wild relative *C. scarabaeoides* as a female parent. The resultant F_1 plant was partial male-sterile and in F_2 a number of male-sterile segregants were recovered. Subsequently, a perfect male-sterile maintainer line ICPL 288 was also identified. The fertility restoration of this male-sterile line was also found among fertile F_2 segregants. This male-sterile source was used in developing experimental hybrids in Gujarat state of India.

Observation date	Progeny number									
	82		83		90		86		149	
	S	F	S	F	S	F	S	F	S	F
4 September	22	1	21	0	23	0	21	5	20	0
22 September	18	5	6	15	4	19	0	26	11	9
17 October	4	19	1	20	2	21	0	26	2	18
14 November	4	19	2	19	2	21	0	26	2	18
16 February	22	1	21	0	23	0	19	5	20	0
14 March	22	1	21	0	23	0	19	4	19	0
Pods plant^{-1} in converted fertile plants	76–201		97–188		147–260		87–191		126–209	

Table 2: Changes in the proportions of male-sterile (S) and male-fertile (F) plants in the environment-sensitive lines selected from ICPA 85010

S, sterile; F, fertile.

Saxena and Kumar (2003) also crossed *C. scarabaeoides* as a female parent with four pigeonpea cultivars. Among F$_1$s, a progeny derived from cross *C. scarabaeoides* × ICPL 88039 was completely male-sterile. To stabilize this source of male-sterility, backcrosses were made with ICPL 88039 as recurrent parent and all the plants in BC$_1$F$_1$ through BC$_6$ F$_1$ generations were male-sterile. They also reported eight fertility restorers and six male-sterility maintainers. This allowed breeding of genetically diverse hybrids for different cropping systems. Saxena (2008) reported that fertility restoration in hybrids involving this CMS was not perfect and a large variation (50–95%) was observed for pollen fertility. This variation could be due to differential inter-genomic or cytoplasmic–genomic interactions. Abdalla and Hermsen (1972) opined that polymorphism, arising due to differential genes, can also yield inconsistent expressions of both male-sterility and fertility restoration.

CMS with *Cajanus volubilis* (Blanco) Blanco (A$_3$) cytoplasm

Wanjari et al. (1999) selected a number of male-sterile segregants with maternal inheritance from a cross involving *C. volubilis* and a cultivated type. These selections, however, could not be used in any hybrid breeding programme due to lack of fertility restoring genotypes.

CMS with *Cajanus cajanifolius* (Hains) van der Maesen comb. nov. (A$_4$) cytoplasm

Rathnaswamy et al. (1999) unsuccessfully attempted to breed a CMS line by crossing *C. cajanifolius* as male parent with a GMS line as female parent. All the progeny from this cross were male fertile. Saxena et al. (2005) crossed ICPW 29, an accession of *C. cajanifolius*, a wild relative of pigeonpea, as female parent with pigeonpea line ICPL 28. According to De (1974), *C. cajanifolius* resembles with cultivated types in most morphological traits and differs by only a solitary gene. The inter-specific F$_1$ hybrid plants grown in 2001 expressed variable extents of pollen sterility and one plant with 60% pollen sterility was backcrossed to ICPL 28. This was followed by six backcrosses to substitute the nuclear genome of wild species with that of the cultivated type. This substitution led to enhanced male-sterility that was fully maintained by its recurrent pigeonpea parent. This male-sterile source is the best among those identified so far and it was designated as ICPA 2039. It was found to be highly stable male-sterile line across environments and years (Dalvi et al. 2008a, Saxena

2008) and never showed any morphological deformity. To develop diverse pigeonpea hybrids this male-sterile source has now been transferred into a number of genetic backgrounds.

CMS with *Cajanus cajan* (L.) Millsp. (A$_5$) cytoplasm

Rathnaswamy et al. (1999) crossed a GMS line with *Cajanus acutifolius* (F.V. Muell.) van der Maesen comb. nov. as male parent and all the F$_1$ plants were male fertile. Mallikarjuna and Saxena (2002) crossed *C. acutifolius* as a female parent with pigeonpea accession ICP 1140 with only 1.5% pod set. The use of gibberellic acid (at 50 mg/l) in backcrosses enhanced the pod set to 6% but the seeds, thus obtained, were under developed and failed to germinate. To overcome this problem, the developing embryos were rescued and successfully cultured in artificial media (Mallikarjuna and Moss 1995). Encouraged with the success of embryo rescue technology, Malikarjuna and Saxena (2005) again crossed six pigeonpea cultivars as female parent with two accessions (ICPW 15613, ICPW 15605) of *C. acutifolius*. The F$_1$s involving pigeonpea lines ICPL 85010, ICPL 85030 and ICPL 88014 produced a few male-steriles with some plants exhibiting up to 100% pollen sterility. The anthers of these male-sterile plants were shrunken and pale yellow in colour. Such male-steriles maintained their sterility when crossed to their respective wild relative accessions. Most of the cultivated accessions when crossed to these male-steriles restored the male fertility of the plants. An exception to this was HPL 24, where F$_1$ progeny produced both male-sterile and fertile plants. This suggests the presence of both *fr* and *Fr* genes in its nuclear genome. Further backcrossing with this line and selection for pollen sterility helped in stabilizing the male-sterility (K. B. Saxena, Unpublished data). Interestingly, HPL 24 was bred from a cross involving *C. sericeus*, another wild species (Saxena 2008), and this suggested that besides *C. acutifolius* the *fr* genes may also be present in *C. sericeus*.

CMS with *Cajanus lineatus* (W. & A.) van der Maesen comb. nov. (A$_6$) cytoplasm

In 2002 rainy season, a naturally out-crossed partial male-sterile plant was observed in an open-pollinated population of *C. lineatus* (K. B. Saxena, Unpublished data) and the morphology of this plant was very different from rest of the population. The vegetative cuttings of this plant were raised in a glasshouse and out of five cuttings planted only two survived and the plants were found to be male-sterile. These were

crossed with pigeonpea line ICPL 99044 and produced normal pod set. The F_1 plants grown in 2004 season were partial male-sterile. Back-crosses (BC_1F_1) were made with ICPL 99044 and out of 20 plants grown five were partial male-sterile. In BC_4F_1 generation, 167 plants were examined for pollen viability and it ranged from 92% to 100%. The plants showing 100% male-sterility were crossed with four pigeonpea lines in 2008 season. At present this CMS source is in BC_5F_1 stage with perfect male-sterility maintenance system available.

CMS with *Cajanus platycarpus* (Benth.) van der Maesen comb. nov. (A_7) cytoplasm

Cajanus platycarpus, a wild species in the tertiary gene pool of pigeonpea, is cross incompatible with cultivated types and, therefore, hormone-aided pollinations coupled with embryo rescue techniques were employed to obtain viable F_1 and BC_1F_1 progeny (Mallikarjuna et al. 2006). In BC_2F_1 genera-tion, a progeny (BC_2-E) with low pollen fertility was selected. Within this progeny two plants with 100% pollen sterility were selected and crossed with a set of pigeonpea cultivars. The examination of their F_1s showed that the hybrid involving cultivar 'ICPL 85010' maintained complete male-sterility, whereas cultivars 'ICPL 88014' and 'ICP 14444' restored male fertility. The detailed studies on this new CMS source are in progress.

Blockages in Microsporogenesis of Male-Sterile Genotypes

The differentiation of meristematic tissues into pollen mother cell (PMC) is brought about by a series of bio-chemical events led by inductive photoperiod and thermal changes. This is followed by various development changes to produce pollen grains. In the determination of male-sterility in crop plants, the anther wall and in particular the tapetum, plays an important role of producing and transporting critical enzymes, hormones and nutrients that are essential for the growth of PMCs and any abnormality in the anther wall development leads to the production of defective pollen grains. Vasil (1967) deliberated that during the process of meiosis any abnormality in the supply of nutrients generally leads to aberrant outputs such as large and more number of PMCs. Abnormal vacuolization or fusion of cells into multi-nuclear syncytia, or degeneration of the tapetal layer leads to the abnormal development and separation of PMCs. The normal development of PMCs in general is arrested either premeiotic, during meiosis or in postmeiotic stages of growth.

Reddy et al. (1978) reported identification of the first GMS system in pigeonpea. The cytological studies on the fertile and sterile siblings showed that the microsporogenesis in the two genotypes was similar up to tetrad formation stage. The differences between the two emerged when the tetrads in the male-sterile plants failed to be released and leading to degeneration of tetrads through vacuolation. The tapetum continued to persist even when the tetrads degenerated. On the contrary, in the fertile plants, tapetum began to degenerate during the formation of tetrad and disappeared during male gametophyte development. In case of male-sterility, the callose is synthesized because of the presence of high concentrations of cellular calcium (Worrall et al. 1992). Ketti et al. (1994) conducted further studies on the persistence of callose and tapetum in the ms_1 type of male-sterility and reported the accumulation of callose and persistent tapetum during post-meiotic stages. They further deliberated that a gradual reduction in the concentration of polysaccharides and RNA proteins in the tetrads were responsible for disorientation of cytoplasm leading to malnutrition and poor tetrad growth.

Saxena et al. (1983) reported male-sterility, where the anthers were brown and shrivelled. Dundas et al. (1981) revealed that the degeneration of microspores occurred at the tetrad stage through rupturing of nuclear membrane and collapse of the outer wall resulting.

Dundas et al. (1982) while reporting a new source of GMS observed that in the male-sterile plants the PMCs count was almost double than their fertile counterparts. The abnormal enlargement of PMCs and their number was associated with the failure of adjacent PMC walls to separate. The breakdown of microsporogensis of this male-sterile occurred at prophase I. The delayed and incomplete anther wall development appeared to be responsible for PMC degeneration. Similar observations were also reported by Murthi and Weaver (1974) in cotton. Frankel and Galum (1977) suggested that early breakdown of microsporogenesis was associated with varying degrees of impairment of fertility of female gametes. Contrary to this theory, the female fertility in this male-sterile line was normal.

In the GMS reported by Saxena et al. (1983), the develop-ment of sporogeneous tissues and young PMCs was similar in the male-fertile and sterile plants. In the male-sterile plants, however, PMC degeneration occurred at young tetrad stage with the rupturing of nuclear membrane and collapse of outer cell walls. The vacuoles developed in the tapetal cells metaphase I and by tetrad stage the entire cell gets vacuolated. In this case, the precocious degeneration of tapetum ending its role as a nutrient source for PMCs (Echlin 1971) could be responsible for tetrad breakdown. Similar results were also reported by Kaul and Singh (1966) in *Hordeum vulgare*; Overman and Warmke (1972) in *Sorghum vulgare* and Reddy and Reddi (1974) in *Pennisetum typhoides*.

In all the three GMS systems, the blockages in the microsporogenesis occured at different stages of development which also determined their anther morphology. Studies of Saxena et al. (1983) and Saxena and Kumar (2001) showed that if an individual plant carries two male-sterility inducing genes, then the one which expresses first and hinders the normal process of microsporogenesis, determines the pheno-type of the anthers and the other genes become redundant as far as their expression is concerned. Cytological examination of sparse pollen producing flowers revealed that their tetrad formation was normal but soon after this, only a portion of microscopores collapsed. Further, the locules of anthers within individual flowers varied in the proportions of microspore degeneration (Saxena et al. 1981). The cause of this partial breakdown of microsporogenesis could not be ascertained.

Ariyanayagam et al. (1995) working with *C. sericeus*-derived CMS lines, reported that meiosis in the male-sterile plants proceeded normally until the release of microspores and this was followed by vacuolation and degeneration of protoplasm. Cytological investigations with *C. acutifolius*-derived CMS showed that the process of meiosis in the male-sterile plants proceeded normally till the onset of tetrad stage (Malikarjuna and Saxena 2005), but their further growth was arrested and the tetrads remained inside the tapetum layer. This resulted in the loss of cell contents and collapse of the process of microsporogenesis.

A detailed study by Malikarjuna and Kalpana (2004) identified two different kinds of male-sterile plants in a cross involving a cultivated pigeonpea as female parent and *C. acutifolius* as male parent. These two male-sterile variants had different anther morphology. In type I, the anthers were shrivelled with brown colour, while in type II male-steriles, the plants had pale white shrivelled anthers. These variants also differed in their microsporogenesis. The PMCs of type I male-sterile plants remained in prophase stage and subsequent processes of meiosis were arrested. The PMCs enlarged normally and once nucleus grew, further cell division did not take place. In these plants, persistence of tapetum was also observed. In type II plants, the anthers were translucent and microsporogenesis continued up to tetrad stage but the tetrads failed to separate and produce pollen grains. This was followed by collapse of anther development process, a sort of postmeiotic arrest of microspore development. Dalvi et al. (2008b) studied cytogenetics of A_4 CMS and reported an early breakdown of tapetum. In these plants, the anthers were under-developed and the male-sterility expressed at tetrad stage, where the tetrad wall failed to degenerate and resulted in the degeneration of its contents.

It can be concluded that GMS in pigeonpea occurs due to two primary reasons. The first process is characterized by the development of brown and shrivelled anthers followed by premeiotic breakdown of PMCs. In the other process, the anthers are pale white or translucent accompanied by postmeiotic breakdown of PMCs.

It is now well understood that the CMS trait is expressed due to impairment of pollen formation processes that result from interaction of the nuclear and the mitochondrial genomes. Pollen maturation requires great amounts of energy (Zhao et al. 2000). It is well known that there is many fold increase in the number of mitochondria in the tapetal tissue and PMCs during pollen development. In sugarbeet and wheat, low temperatures cause CMS like microspore disturbances as microspores and tapetum cells are more sensitive than the female reproductive organs and oxidative processes are responsible for this development (Kuranouchi et al. 2000). It is also believed that the mitochondria have a major role to play in the expression of CMS trait. In pigeonpea, there is only one report (Sivaramakrishnan et al. 2002) that deals with the assessment of mitochondrial genome of the CMS plants.

Inheritance of Male-Sterility and Fertility Restoration Systems

With one exception, all the reported sources of GMS are controlled by a single recessive gene pair. Reddy et al. (1978) designated the GMS gene as ms_1, while Saxena et al. (1983) reported a non-allelic relationship between ms_1 and ms_2 genes. They also reported that during microsporogenesis the ms_2 is expressed at an earlier stage than that of ms_1 gene. The male-sterility reported within ICPL 85010 population (Saxena and Kumar 2001), was also controlled by a single recessive gene (ms_3) and it was also non-allelic to ms_1 and ms_2 genes. They further reported that all the three male-sterility genes were independent and when present within a plant system, expressed independently at different stages of microsporogenesis. The first to express is ms_2, followed by ms_3, and finally ms_1 gene. The translucent type of GMS reported by Verulkar and Singh (1997) was also controlled by a single recessive gene but its allelic relationship with ms_1 which also has translucent anthers

was not studied. Saxena et al. (1981) reported single recessive genetic control of sparse pollen production in pigeonpea.

Among various CMS sources reported, the genetics has been reported for only A_4 type of CMS. Dalvi et al. (2008a) studied genetics of fertility restoration in five crosses. Of these, in three crosses a single dominant gene, while in one cross two dominant genes with duplicate gene action restored the fertility. In the fifth cross also two dominant genes with complimentary action governed the fertility. Further investigation into the origin of fertility restoring lines showed that these *Fr* genes were randomly distributed in the germplasm.

Effect of Environment on Male-Sterility Systems

Ariyanayagam et al. (1995) while attempting to breed a CMS system at ICRISAT (17°N) using multiple genome transfer approach identified a progeny where the male-sterile segregants, expressed 100% pollen sterility from of March to June but it started producing small quantities of pollen grains in July. They attributed it to the reduction in mean temperature and rise in humidity. Saxena (2009) reported the results of a detailed study at ICRISAT with CMS lines derived from *C. sericeus* under a selfing cage (to avoid the entry of pollen-carrying insects) and reported that the process of conversion of male-sterile plants to male fertility started at the end of September and continued up to middle of November. Also, it was observed that there was a genetic variation for this trait among and within progeny. Such converted male-fertile plants reverted back to male-sterility in the month of February (Table 2). These observations suggested that shortening of daylengths and reduction in temperatures induced male fertility, while high temperatures and longer days maintained male-sterility. The detailed studies on this subject are in progress.

Molecular Characterization of Male-Sterile Lines

Although the CMS-based hybrid pigeonpea technology is now ready for use with high yield potential, their genetic and molecular basis is yet to be investigated. Recent advances in pigeonpea genomics as a part of International Pigeonpea Genomics Initiative (Varshney et al. 2009) offer a good scope to characterize CMS (A-) lines along with their maintainers (B-) and restorer (R-) lines at molecular level. The identification of molecular markers associated with fertility restoring gene(s) is also a potential research areas under consideration.

Recently, a number of DNA marker systems such as restriction fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD), diversity arrays technology (DArT) and simple sequence repeats (SSRs) have been used for pigeonpea genetic diversity analysis (Nadimpalli et al. 1993, Ratanparkhe et al. 1995, Burns et al. 2001, Yang et al. 2006, Odeny et al. 2009, Saxena et al. 2009a,b) but only two reports are available on the characterization of A-, B- and R-lines. Sivaramakrishnan et al. (2002) compared RFLP patterns using maize mitochondrial DNA-specific probes in three pigeonpea CMS progeny with A_2 cytoplasm and two GMS lines. The results confirmed the maternal inheritance of male-sterility. Similarly, RAPD markers (Souframanien et al. 2003) were used for the identification of pigeonpea CMS lines derived from two crosses (*C. scarabaeoides* × *C. cajan* and *C. sericeus* × *C. cajan*). They also reported adequate polymorphism to differentiate among A-, B- and R-lines with

certain random primers. Recently, SSR genotyping of 37 A-, 38 B- and 84 R-lines was conducted at ICRISAT with 148 SSR markers (R. K. Saxena, Unpublished data). The main aim of this work was to obtain finger printing data of promising A-, B- and R-lines and to select parents for developing mapping populations for tagging the fertility restoration (*Fr*) genes in pigeonpea (Varshney et al. 2009). This study has provided a total of 41 markers polymorphic across 159 lines used with an average of 3.1 alleles and polymorphism information content (PIC) value of 0.41 per marker. As evident by PIC values, among B-lines (0.39) a relatively higher genetic dissimilarity was observed when compared with A- (0.34) and R- (0.37) lines. Based on these results, eight mapping populations including four F_2 and four back-cross (BC_1F_1) populations are being developed. These populations will be used for genotyping with SSR and DArT markers. The genotyping data along with phenotyping data are likely to identify the markers associated with *Fr* genes in pigeonpea.

As mentioned earlier, CMS is caused by impaired micro-sporogenesis, which results in anthers with abortive pollen. CMS is maternally inherited and expressed through interactions of nuclear genes with cytoplasmic determinants assigned to the mitochondrial genome (Budar and Berthome 2007). Generally, CMS is accompanied by changes in the pattern of plasmid-like DNAs, in expression of several genes and in the mitochondrial genome structure (Zubko 2004). With an objective of identification and expression of such genes, mitochondrial genomes have been investigated in detail in several species such as pepper (Kim and Kim 2006), sugar beet (Satoh et al. 2004), rice (Wang et al. 2006), *Brassica napus* (Carlsson et al. 2007) and maize (Allen et al. 2007), However, it is still unknown which regions of the mitochondrial genome interact with nuclear genes to cause CMS in pigeonpea. In this direction, some efforts have been initiated at ICRISAT, in collaboration with J. Craig Venter Institute (JCVI), USA, to sequence the mitochondrial genomes of ICPA 2039 (A-line), ICPB 2039 (B-line), ICPH 2433 (hybrid) and ICPW 29 (wild species) by using 454/FLX sequencing technology. This study should shed some light on the changes in the pattern and expression of mitochondrial genes that leads to CMS in pigeonpea.

Utilization of Male-Sterility Systems in Pigeonpea Breeding
Population improvement
Broader genetic base and high recombination frequency are the two key parameters that help in breeding for the desired end products. In crops like pigeonpea, the extent of recombi-nation is rather limited due to predominant selfing. This adversely affects the selection efficiency. The use of double, three-way, or composite crosses helps in broadening the genetic base of segregating populations to some extent but

the inherent genetic linkages restrict the desired level of gene reshuffling. To overcome this challenge in the self-pollinated crops, Suneson (1956) and Jensen (1970, 1978) proposed mating schemes among selected genotypes using cumbersome hand pollination procedures. Also at ICRISAT, a dual population breeding scheme using a recessive trait (obtuse leaf) was implemented (Green et al. 1981) in pigeonpea. To facilitate the genetic recombination in pigeonpea, Byth et al. (1981) proposed the use of GMS genotypes and natural out-crossing. Faris (1985) developed six recurrent selection popu-lations at ICRISAT using GMS lines and natural out-crossing for enhancing yield but the gains were discouraging. The success of such population breeding schemes in practical pigeonpea breeding, however, is yet to be established.

Hybrid breeding
GMS-based hybrids
Presence of partial natural out-crossing and development of a stable male-sterility system offered a unique opportunity to exploit hybrid vigour in pigeonpea. Efforts in this direction were initiated at ICRISAT by using the male-sterility system discovered by Reddy et al. (1978). The experimental hybrids demonstrated significant heterosis over control cultivar. Out of 182 GMS hybrids tested at ICRISAT, 59 demonstrated more than 60% yield advantage over the best control. From this programme the world's first pigeonpea hybrid ICPH 8 was released for cultivation in India (Saxena et al. 1992). To the best of our knowledge this is the first ever commercial hybrid in any food legume. In farmers' fields, this hybrid recorded 31–40% yield advantage over the best control. This was followed by the release of five more pigeonpea hybrids. Which exhibited high levels of standard heterosis (Table 3). But, none of these could reach farmers' fields at commercial level and the main hindrance was the large-scale seed production of female parent. As the male-sterility is controlled by a pair of recessive gene (*msms*) and it can only be maintained by crossing it to heterozygous (*Msms*) plants. The progeny of this cross (*msms* × Msms) will segregate in to 50% male-fertile (*Msms*) and 50% male-sterile (*msms*) plants. Therefore, identification of the fertile segregants within female population was primary requirement of large-scale seed production and it was not found commercially viable.

CMS-based hybrids
Soon after achieving the long awaited breakthrough of developing a stable CMS line, a number of experimental hybrids were synthesized and tested. In these hybrids, 25–110% standard heterosis was recorded in station trials. Among these, two hybrids ICPH 2671 and ICPH 2740 were found promising. In multi-location trials, conducted for 4 years ICPH 2740 recorded 35.8% superiority over the control (Fig. 1). During 2009, the best performing hybrid ICPH

Table 3: GMS-based pigeonpea hybrids released in India

| Character	ICPH 8	PPH 4	CoH 1	CoH 2	AKPH 4104	AKPH 2022
Year released	1991	1994	1994	1997	1997	1998
Adaptability	Central Zone	Punjab	Tamil Nadu	Tamil Nadu	Central Zone	Maharashtra
Plant type	Indeterminate	Indeterminate	Indeterminate	Indeterminate	Indeterminate	Indeterminate
Days to maturity	125	137	117	125	135	190
Yield superiority over check (%)	30–41	14	19–22	35	64	25–35
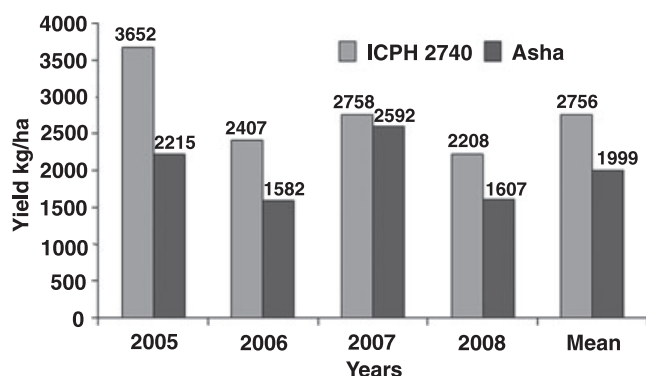
Fig. 1: Performance of hybrid ICPH 2740 over 4 years in 33 locations in India

Table 4: Over all performance of hybrid ICPH 2671 in on-farm trials conducted in India, 2008

State	No. trials	Total area (ha)	Mean yield (kg/ha)		Superiority (%)
			Hybrid	Control	
Maharashtra	782	261	969	717	35
Andhra Pradesh	172	70	642	441	45.6
Karnataka	14	6	1131	951	19
Madhya Pradesh	280	66	1490	1192	25
Total	1248	403	1058	825	28.2

2671, was evaluated in 1248 on-farm trials in four Indian states (Table 4). In these trials ICPH 2671, on average, recorded 28.4% yield advantage over local control. Under intercropping conditions this hybrid was outstanding and when grown in combination with soybean (1 : 5 ratio) and produced the highest yield of 3300 kg/ha was recorded in a large (1.1 ha) field. It appears that the CMS-based hybrids hold a great promise for enhancing the stagnant productivity of pigeonpea.

Summary and Outlook

Pigeonpea is known to have tremendous genetic variability but breeders have not been able to exploit it for the genetic enhancement of yield. In the last 50 years, more than 100 pure line pigeonpea varieties have been released in India alone (Singh et al. 2005), but the national productivity has remained stagnant around at low levels. A number of genetic studies conducted on this subject in pigeonpea revealed that a considerable extent of non-additive genetic variance is also present in the crop which was never utilized in breeding high-yielding cultivars (Saxena and Sharma 1990) and invariably additive genetic variances were utilized for the enhancement of yield and other related traits. Successful breeding of a CMS system has given a golden opportunity to breeders to utilize non-additive genetic variation for increasing pigeonpea productivity.

The Herculean breeding efforts at ICRISAT, spanning over 30 years, culminated with the selection of an excellent CMS system. Breeders have now developed technologies for large-scale seed multiplication of hybrids and their parents. The presence of high level of realized heterosis in farmers' fields has opened the way for commercialization hybrid pigeonpea technology. It is expected that the development of a large number of hybrid combinations will allow selecting specifically adapted hybrids with even high levels of hybrid vigour. These developments have demonstrated that now a long-awaited quantum jump in pigeonpea yields is possible.

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