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Cytogenetic Studies in A_4 Cytoplasmic-Nuclear Male-Sterility System of Pigeonpea

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Efforts were made to study microsporogenesis and genetics of fertility restoration of A_4 cytoplasmic-nuclear male-sterility (CMS) system in pigeonpea. The process of microsporogenesis in the male-sterile (ICPA 2039) and its maintainer (ICPB 2039) plants was normal up to the tetrad formation stage. The tapetal cells in the male-sterile anthers degenerated soon after tetrad formation, resulting in shriveled and degenerated microspores. In the maintainer plants, the tapetal cells were normal and microspores were functional. The breakdown of the tapetum before the completion of microsporogenesis was the major cause for the expression of male sterility in A_4 CMS system. The studies on the inheritance of fertility restoration showed that in 3 crosses, a single dominant gene; in 1 cross, 2 duplicate genes; and in another cross, 2 complimentary genes governed the fertility restoration.

Key words: *CMS, genetics, histology, pigeonpea, tapetum*

Cytoplasmic-nuclear male-sterility (CMS) systems have played an important role in exploiting hybrid vigor for enhancing productivity in field crops as well as in horticultural crops (Saxena 2005). Pigeonpea (*Cajanus cajan* (L.) Millspaugh) is perhaps the first food legume where commercial hybrids are being bred (Saxena et al. 2006; Stakstad 2007). Of the 5 CMS systems developed so far in pigeonpea, the A_4 system derived by crossing *Cajanus cajanifolius*, a wild relative of pigeonpea and a cultivar (Saxena et al. 2005), is the best because it is stable across environments and has a large number of fertility restorers (Saxena et al. 2006). The wild species and cultivar used in these crosses to develop CMS systems were genetically diverse (Remanandan et al. 1988). Using this CMS system, a number of high yielding experimental hybrids have been developed and one such hybrid is under extensive production and agronomic testing in India (Saxena 2007). The objectives were to determine the histological reasons responsible for the manifestation of this male-sterility system and to study the genetics of its fertility restoration.

Materials and Methods

Seeds of the male-sterile line ICPA 2039 and its maintainer ICPB 2039 were planted at the research farm of Marathwada Agricultural University, Parbhani (Maharashtra), India, during the 2005 rainy season (June). Cultural practices recommended to raise a successful crop were followed. Ten flower buds of 3 to 5 mm size were harvested in the early morning hours (0600–0700) from 3 randomly selected plants each from the male-sterile and male-fertile populations. The buds were fixed in Carnoy's fluid (Mallikarjuna and Kalpana 2004). These buds were transferred to 70% ethanol and kept for 24 h and then passed through a series of solutions comprised of rectified spirit, ethanol, and xylol. In each solution, the buds were kept for 30 min. The buds were transferred to paraffin wax and kept at 60 °C for 5–10 min. Transverse sections of 3–5 μm thickness were cut using a rotary microtome. For staining the tissue, sections were passed through a sequence of solutions of haematoxylene, eosin, fast green, rectified spirit, and ethanol with 5- to 7-min treatment in each solution (Culling 1974; Dundas et al. 1981; Kitti et al. 1994). The various microsporogenesis stages were identified under a light microscope (×40 and ×100).

To study the inheritance of fertility restoration, the male-sterile line was crossed with 5 inbred restorers ICP 12320, ICP 11376, HPL 24-63, ICP 10934, and ICP 13991. The F_1 plants of all the crosses were grown at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru during 2005 rainy season (June). The individual F_1 plants were selfed with nylon net bags (0.5 mm) to produce F_2 seeds and 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

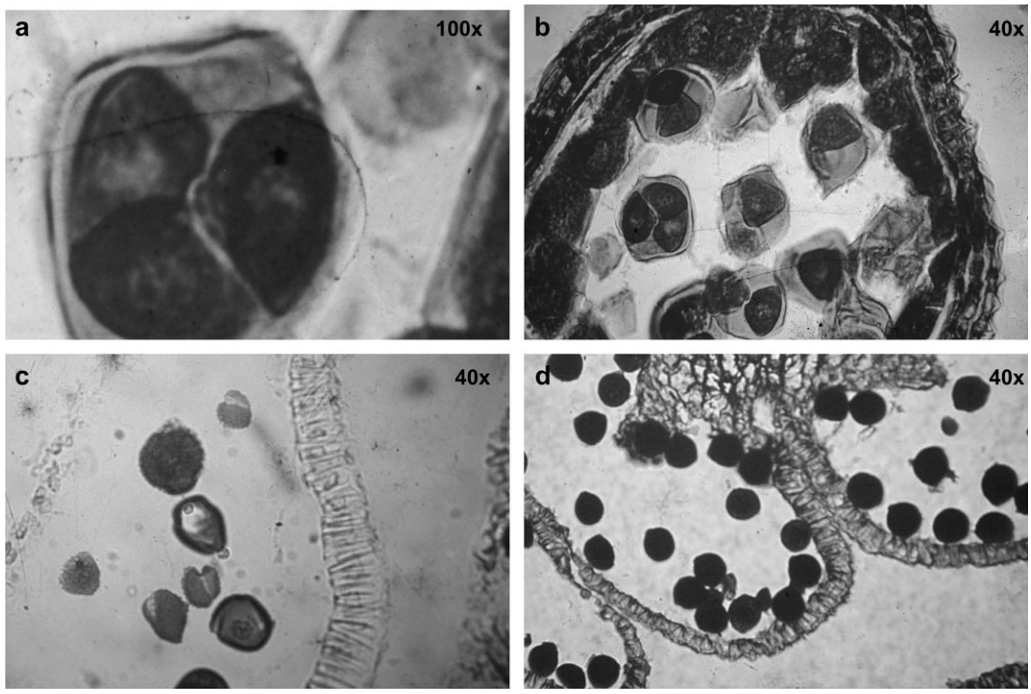

Figure 1. Transverse section of anthers of male-sterile (left) and male-fertile (right) plants of pigeonpea from tetrad stage to pollen grain development stage. (**a, b**) Tetrad stage pollen of male-sterile and male-fertile plants, respectively. (**c, d**) Mature anthers of male-sterile (undeveloped)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

(June) at Patancheru. Data on segregation for male sterility and fertility were recorded in each generation, and Chi-square (χ^2) method was applied to test the goodness of fit for various segregation ratios.

Results and Discussion

In comparison to the fertile plants, the anthers of male-sterile plants were smaller in size and totally devoid of fertile pollen grains. On the contrary, the anthers from the fertile plants contained >99% fertile pollen grains. Pod and seed set on the male-sterile plants after hand pollination and natural outcrossing by insects indicated that the male-sterile plants had excellent female fertility.

Transverse sections of young anthers of similar sizes from both male-sterile and fertile plants showed no differences in the development of sporogenous tissue. The process of microsporogenesis was similar up to the stage of differentiation of pollen mother cells (PMCs) into tetrad formation (Figure 1a,b), but it differed there after. There were no differences between male-sterile and male-fertile anthers for the number of PMCs. Similar observations were reported in the 2 pigeonpea genetic male-sterility systems (Reddy et al. 1978; Dundas et al. 1981). But in another genetic male-sterile line of pigeonpea (Dundas et al. 1982) and in soybean (Palmer et al. 1978), higher numbers of PMCs in male-sterile anthers were reported. The PMCs in the male-sterile plant were shriveled. Early breakdown of the tapetum layer appeared to be the major reason for the manifestation of male sterility. The tapetum layer is known to help in supplying nutrition to the developing PMCs, and due to its early breakdown, the processes of PMC development were terminated, which resulted in male sterility (Figure 1c: shriveled pollen grains).

In case of fertile plants, the process of microsporogenesis proceeded normally and all the anther wall layers were fully developed by the time PMCs had formed. Unlike the male-sterile plants, the tapetum layer in the fertile plants was persistent and it ruptured only after the complete development of pollen grains (Figure 1d: completely developed pollen grains). This facilitated the release of the fully developed pollen grains. Vasil (1967) and Echlin (1971) emphasized the importance of tapetum in the development of microspores. According to Vasil (1967), the anther wall, in particular the tapetum, plays a major role in microsporogenesis by producing and transporting vital enzymes, hormones, and nutritive materials to the PMCs. Hence, the early breakdown of tapetum appeared to be the primary reason for the collapse and abnormal development of PMCs. The process of meiosis in plants is chemically controlled through tapetal secretions, which may influence the development of the sporogenous tissues prior to meiosis and, in particular, the number of meiotic divisions of the archesporial cells to form the normal number of PMCs (Vasil 1967). Gates (1911) in *Oenothera* (*Oenothera gigas*) and Yogesha (1991) in rice (*Oryza sativa*) also reported that the premature breakdown of tapetum was the primary cause for male sterility.

Table 1. Segregation for male sterility and fertility in F_1, F_2, and BC_1F_1 populations of crosses involving a CMS line ICPA 2039 and 5 fertility restorers in pigeonpea

	Number of plants													
	F_1 generation		F_2 generation						BC_1F_1 generation					
Cross	Fertile	Sterile	Total	Fertile	Sterile	Expected χ^2	Calculated χ^2	*P* (0.01)	Total	Fertile	Sterile	Expected χ^2	Calculated χ^2	*P* (0.01)
ICPA 2039 × ICP 12320	27	0	428	317	111	3:1	0.20	0.66	103	42	61	1:1	3.5	0.06
ICPA 2039 × ICP 11376	30	0	430	312	118	3:1	1.37	0.24	158	68	90	1:1	3.06	0.08
ICPA 2039 × HPL 24-63	25	0	471	373	98	3:1	4.42	0.04	115	68	47	1:1	3.83	0.05
Pooled	82	0	1329	1002	327	3:1	0.11	0.74	376	178	198	1:1	1.06	0.3
ICPA 2039 × ICP 10650	32	0	179	166	13	15:1	0.31	0.58	108	78	30	3:1	0.44	0.5
ICPA 2039 × ICP 13991	30	0	518	275	243	9:7	2.10	0.15	112	37	75	1:3	3.86	0.04

The exact *P* values obtained by the computer program are added in this table.

Genetics of Fertility Restoration

All the F_1 plants in 5 crosses were fully fertile indicating the dominance of fertility restoring genes (Table 1). The F_2 and BC_1F_1 progenies of the crosses involving A_4 CMS line segregated for male sterility and fertility (Table 1). Among the 5 crosses studied, 3 (ICPA 2039 × ICP 12320 [χ^2 = 0.20, P = 0.66], ICPA 2039 × ICP 11376 [χ^2 = 1.37, P = 0.24], and ICPA 2039 × HPL 24-63 [χ^2 = 4.42, P = 0.04]) segregated in a ratio of 3 fertile:1 sterile in F_2 generation, where as in the backcross generation a segregation of 1 fertile:1 sterile was observed (pooled χ^2 = 1.06, P = 0.30). This indicated the monogenic dominant nature of a single fertility restoring gene. The pooled values over the 3 crosses also exhibited goodness of fit for 3 fertile:1 sterile (χ^2 = 0.11, P = 0.74) ratio in the F_2 generation and 1 fertile:1 sterile (χ^2 = 1.06, P = 0.30) in backcross generation. The F_2 and backcross populations of cross ICPA 2039 × ICP 10650 segregated in the ratio of 15 fertile:1 sterile (χ^2 = 0.31, P = 0.58) and 3 fertile:1 sterile (χ^2 = 0.44, P = 0.50), respectively. This suggested the involvement of 2 dominant genes with duplicate gene action. The other cross (ICPA 2039 × ICP 13991) segregated in a ratio of 9 fertile:7 sterile (χ^2 = 2.10, P = 0.15) in the F_2 generation and 1 fertile:3 sterile (χ^2 = 3.86, P = 0.04) in BC_1F_1 generation indicating the presence of 2 complimentary genes for restoring the fertility of male-sterile line. Jan et al. (2002) also reported the presence of 2 complementary genes for fertility restoration in sunflower CMS lines. The female parent used in our 5 crosses was the same but the pollen parents were considerably diverse in origin. ICP 12320 was collected from Pakistan, ICP 11376 is from Nepal, and HPL 24-63 is an advanced breeding line derived from cultivar Baigani × *Cajanus scarabaeoides* cross. In all the 3 parents, a single dominant gene governed the fertility. The other 2 lines, ICP 10650 and ICP 13991, originated from India and Bangladesh, respectively, and these lines had 2 dominant genes for fertility restoration but with different modes of action. From the limited data available, it is not possible to draw definite conclusions about the relationships among these genes. Studies to answer this question are in progress at ICRISAT. In the present study, we used bulk pollen form the male parent and this might have resulted in the difference in BC_1F_1 segregation. Some of the ratios were not fit for the considered segregation. Plantwise crosses are recommended to avoid such anomalies. Use of molecular genetic methods were suggested by Pakozdi et al. (2002) in pepper (*Capsicum annuum* L.) for identification of perfect fertility restorers, such efforts are advisable for easy identification of fertility restorers and male-sterility maintainers.

Conclusions

The studies on microsporogenesis in A_4 CMS line ICPA 2039 revealed that the restriction in the supply of nutrients to developing PMCs caused by breakdown of the tapetum was responsible for producing male-sterile anthers. The inheritance studies revealed that in 3 crosses, 1 dominant gene and in the 2 other crosses, 2 dominant genes, one with complimentary gene action and another with duplicate gene action governed the fertility restoration of A_4 CMS line ICPA 2039. The relationship between these 2 genes was not studied.

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References

Culling CFA. 1974. Handbook of histopathology and histological techniques. 3rd ed, London: Butterworth and Co Ltd. p. 29–221.

Dundas IS, Saxena KB, Byth DE. 1981. Microsporogenesis and anther wall development in male-sterile and fertile lines of pigeonpea (*Cajanus cajan* (L.) Millsp.). Euphytica. 30:431–435.

Dundas IS, Saxena KB, Byth DE. 1982. Pollen mother cell and anther wall development in a photoperiod-insensitive male-sterile mutant of pigeonpea [*Cajanus cajan* (L.) Millsp.]. Euphytica. 31:371–375.

Echlin P. 1971. The role of tapetum during microsporogenesis of Angiosperm. In: Heslop–Harrison J, editor. Pollen development and physiology. London: Butterworths. p. 41–61.

Gates RR. 1911. Pollen formation in *Oenothera gigas*. Ann Bot. 25:909–940.

Jan CC, Zhang TX, Miller JF, Fick GN. 2002. Inheritance of fertility restoration for two cytoplasmic male sterility sources of *Helianthus pauciflorus* (*rigidus*) Nutt. Crop Sci. 42:1873–1875.

Kitti RY, Giddanavar HS, Naik S. 1994. Persistence of callose and tapetum in the microsporogenesis of genic male-sterile *Cajanus cajan* (L.) Millsp. with well formed endothecium. Cytologia. 59:65–72.

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

Pakozdi K, Taller J, Alfoldi Z, Hirata Y. 2002. Papper (*Capsicum annuum* L.) cytoplasmic male sterility. J Central European Agric. 3:149–158.

Palmer RG, Albertsen MC, Heer H. 1978. Pollen production in soybeans with respect to genotype, environment and stamen position. Euphytica. 27:427–433.

Reddy BVS, Green JM, Bisen SS. 1978. Genetic male-sterility in pigeonpea. Crop Sci. 18:362–364.

Remanandan P, Sastry DVSSR, Mengesha HM. 1988. ICRISAT pigeonpea germplasm catalog: passport information. Andra Pradesh (India): International Crops Research Institute for the Semi-arid Tropics.

Saxena KB. 2005. Opportunities for exploiting hybrid vigor in grain legumes for increasing yield and adaptation—a success story of pigeonpea. ICRISAT Paper presented in 7th Annual Symposium of the Department of Agriculture; 2005 Sep 29–30; Gannoruwa, Sri Lanka. p. 59–76.

Saxena KB. 2007. ICPH 2671: a CMS-based high-yielding pigeonpea hybrid. Information brochure. Andra Pradesh (India): International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). p. 4.

Saxena KB, Kumar RV, Latha KM, Dalvi VA. 2006. Commercial pigeonpea hybrids are just a few steps away. Indian J Pulses Res. 19:7–16.

Saxena KB, Kumar RV, Srivastava N, Shiying B. 2005. A cytoplasmic-genic male-sterility system derived from a cross between *Cajanus cajanifolius* and *Cajanus cajan*. Euphytica. 145:291–296.

Stakstad E. 2007. The plant breeder and the pea. Science. 316:196–197.

Vasil IK. 1967. Physiology and cytology of anther development. Biol Rev Camb Philos Soc. 42:327–373.

Yogesha HS. 1991. Studies on adaptability of CMS lines, fertility restoration, causes of male-sterility and heterosis in rice [PhD thesis]. [Bangalore (India)]: University of Agricultural Sciences. p. 235.

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