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Studies on inheritance of male-sterility in *Cajanus cajan* × *C. acutifolius* crosses

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ABSTRACT

Among food legumes, the first ever commercial hybrid breeding technology was developed in pigeonpea (*Cajanus cajan* (L.) Millsp.). For a long-term sustainability of hybrids both genetic as well as cytoplasmic diversity of the hybrid parents is essential. At present A_2 and A_4 CMS systems are being used in hybrid pigeonpea breeding. In this paper a case of maternal inheritance of male-sterility conditioned by the cytoplasm of cultivated pigeonpea is reported. This study was made in crosses involving cultivated genotypes as female parents and a wild relative of pigeonpea, *Cajanus acutifolius*, as male parent. The segregation data in F_1, BC_1F_1, and BC_2F_1 generations established maternal inheritance of male-sterility. This source of CMS, when stabilized through backcrossing and selection to a productive recurrent parent, will provide much needed additional cytoplasmic diversity for the future hybrid breeding programs.

Keywords: *Cajanus acutifolius,* Fertility restoration, Male sterility, Maternal inheritance, Pigeonpea

Over centuries the crop growing environments have gone through vast changes leading to many significant changes in cropping systems, varieties, nature and extent of biotic and abiotic stresses,and market preferred traits. Plant breeding has played an important role in enhancing and sustaining agricultural productivity by developing new adapted varieties and hybrids to meet the challenges of climate change. This has been possible by following two basic principles of plant breeding, namely, creation of genetic variability and selection of the fittest individuals. In pigeonpea (*Cajanus cajan* (L.) Millsp.), a food legume of tropics and sub-tropics, the cytoplasmic nuclear male-sterility(CMS) based hybrid technology is new and the hybrids have shown significant advantage over the leading local cultivars in farmers' fields (Saxena and Nadarajan, 2010). For sustaining the yield gains of hybrid technology in pigeonpea, it is essential to develop hybrids with diverse cytoplasmic and nuclear genome complements. In this direction, serious attempts were made at ICRISAT by developing six sources of CMS systems (Saxena *et al.,* 2010) derived from different wild relatives of pigeonpea; but only A_2 (*C. scarabaeoides*) and A_4 (*C. cajanifolius*) are in use indicating limited cytoplasmic diversity.This paper discusses the efforts made to develop a new CMS system using the cytoplasm of cultivated pigeonpea. *C. acutifolius* is a native to Australia and it is generally found growing in the wild habitats of dry, sandy or rocky soils of Western and Northern parts of the country (van der Maesen, 1986). It is an erect or spreading shrub with 50 to 150 cm height. Its flowers are predominantly yellow. The seeds of *C. acutifolius* are round, plump, black or brown with dark mottles and a prominent strophiole. Pods are about 15-20 mm long and 6-8 mm wide, flat with velvety hairs and on average each pod contains 2-3 seeds. Leaflets are of green color and have short silvery hairs. The pigeonpea cultivars used in this study were ICP 28 and ICPL 85030 and both of them are determinate in growth habit, short in height (<100 cm) and early (100-120 days) in maturity. Like other members of this genus,*Cajanus acutifolius* also has a chromosome complement of 2n =2x = 22; but it has a distinct karyotype with a very low (1.44) ratio between the longest and the shortest chromosomes (Ohri and Singh, 2002).

MATERIALS AND METHODS

Four accessions (ICPW 2, ICPW 6, ICPW 9, ICPW 12) of *Cajanus acutifolius* (F. Muell.) Maesen (synonyms: *Atylosia acutifolia; Rhincosia acutifolia*), a wild relative of pigeonpea, and two cultivars were selected for this study.To facilitate the germination, the surface of *C. acutifolius* seeds was scarified with a sharp blade and sown along with pigeonpea cultivars in 30 cm diameter plastic pots using a mixture of sand (1 part) and Alfisols (4 parts).The pots were kept in a glass house at 25+3.5^{0}C and irrigated twice weekly. At flowering,young but unopened floral buds of pigeonpea cultivars were emasculated manually and the pollinations were performed between 0900- 1200 hrs with fresh pollen collected from *C. acutifolius* plants (Table 1). The harvested crossed seeds were small, dark colored, and shriveled with only 60-70% germination. All the F_1 plants exhibited growth habit that was more or less intermediate between the two parents used in the crosses. Every F_1 plant of each cross was examined for its pollen production and its viability. For this purpose, 8-10 fully-grown floral buds were harvested from different parts of each plant and their pollen was extracted by squashing the anthers on a slide and staining the contents with 2% aceto-carmine solution; and subsequently each slide was examined under10X magnifications. The plants with pollen viability > 98% (similar to pigeonpea parents) were considered male-fertile; while plants with no pollen grains were classified as male-sterile. These male-sterile plants were used for backcrossing with respective cultivated pigeonpea parents and their progenies were also studied for pollen viability. The plants with varying pollen viability were classified as partial

male-fertile.The male plants used in crosses were bagged to produce pure seeds.

RESULTS AND DISCUSSION

In this study a limited number of pollinations were attempted using cultivated pigeonpea as female parent and none of the combinations were found cross incompatible (Table 1). It was further noted that the crosses made on ICP 28 had greater chance of success (mean = 78%) in comparison to that on ICPL 85030 (mean = 59%). The crosses of ICP 28 with wild species accessions ICPW 6 and ICPW 12 were better with 96% and 100% pod set, respectively. The results suggested relatively favourable genome-plasmon interaction between ICP 28 and the two C. *acutifolius* accessions.

Examination of F_1 plants revealed that none of the crosses was fully male-fertile, suggesting that the cultivated cytoplasm failed to fully complement the nuclear genome of *C. acutifolius*. This may be due to unfavorable interaction of *C. cajan* mitochondrial genome with nuclear genome of *C. acutifolius* leading to invalid microsporogenesis that was expressed in the form of male-sterility. The F_1 observations further suggested the absence of dominant fertility restoring genes in the four wild species accessions. Among *C. acutifolius* accessionsused as male parent,ICPW 6 and ICPW 12 produced greater proportions of male-sterile plants when crossed to pigeonpea genotype ICP 28 (Table 2). Dundas *et al.* (1986) also reported that most of the hybrids involving *Atylosia* (=*Cajanus*) *acutifolius* showed high (up to 79%) pollen sterility, whereas the hybrids involving other species showed moderate levels of pollen sterility (28-38%). Mallikarjuna and Saxena (2005) crossed six pigeonpea cultivars with two *C. acutifolius* accessions as male parent and found that the crosses segregated into male-sterile and fertile plants; and their proportions varied from cross to cross. The observations in the present study showed significant intra-species genetic variability, but ICPW 6 and ICPW 12, as a group, may be genetically closer to each other, but different from ICPW 2 and ICPW 9. Also, ICPW 6 and ICPW 12 may be genetically more distant from ICP 28 compared to ICPW 2 and ICPW 9. Ratnaswamy *et al.* (1999) crossed *C.acutifolius* as a female parent with a pigeonpea line MS Co 5 and reported partial male-fertility in all the F_1 plants. Joginder *et al.* (2004) also reported various meiotic abnormalities associated with varying degrees of male-sterility in the hybrid plants of a cross between cultivated pigeonpea (female) and *C. acutifolius* (male).

The expression of male-sterility in the hybrid plants suggested that nuclear genes of the wild species influenced the mitochondrial genes of the cultivated types and produced progenies with abnormal microsporogenesis resulting in male-sterility. Dundas (1990) also reported that Australian wild relatives of pigeonpea tended to have high levels of meiotic abnormalities and greater genetic differences for heritable characters than reported in hybrids involving pigeonpea and Indian wild species.

Examination of anthers of the male- sterile hybrid plants in the present study revealed the presence of two anther types in each cross and both the types were devoid of pollen grains. These observations were similar to those made by Mallikarjuna and Kalpana (2004) who reported that the Type I male-sterile plants had brown and shriveled anthers. In these plants the process of microsporogenesis turned invalid at pre-meiotic stage. On the contrary, in Type II male-sterile plants the anthers were fully grown and white (translucent).In such plants the microsporogenesis ceased at post meiotic stage,soon after the formation of tetrads. Xu *et al.*(1995) demonstrated that anthers with different morphology have different restriction fragment length polymorphism (RFLP) patterns which can be distinguished using specific mDNA sequence.

In BC_1 F_1 and BC_2F_1 generations all the crosses segregated for male-sterility and fertility but no trend was observed among the progenies (Table 2),. The progenies of

Table1: Pollination record and number of crossed seeds obtained in eight inter-specific crosses

Parameter	Male / Female	ICPW 2	ICPW 6	ICPW 9	ICPW12	Mean
Pollinations (no.)	ICP 28	45	23	58	29	39
	ICPL 85030	12	20	18	20	18
	Mean	28.5	21.5	38	24.5	28.5
Pods (no.)	ICP 28	21	22	40	29	28
	ICPL 85030	4	10	15	14	11
	Mean	12.5	16	27.5	21.5	19.5
% Success	ICP 28	47	96	69	100	78
	ICPL 85030	33	50	83	70	59
	Mean	40	73	76	85	68.5
Seeds (no.)	ICP 28	43	50	85	74	63
	ICPL 85030	11	30	49	48	35
	Mean	27	40	67	61	48.8

Table 2: Results of pollen study in BC_1F_1 and BC_2F_1 generations in eight inter-specific crosses.

Cross	Number of F_1 plants				Number of BC_1F_1 plants				Number of BC_2F_1 plants			
	Total	Sterile	Fertile	Partial fertile	Total	Sterile	Fertile	Partial fertile	Total	Sterile	Fertile	Partial fertile
ICP 28 x ICPW 2	20	5	0	15	51	15	0	36	49	7	2	40
		(25)	(0)	(75)		(29.4)	(0)	(70.6)		(14.3)	(4.1)	(81.6)
ICP 28 x ICPW 6	20	15	0	5	47	8	0	39	21	4	0	17
		(75)	(0)	(25)		(17)	(0)	(83)		(19)	(0)	(81)
ICP 28 x ICPW 9	14	8	0	6	12	2	0	10	5	2	0	3
		(57)	(0)	(43)		(16.7)	(0)	(83.3)		(40)	(0)	(60)
ICP 28 x ICPW 12	17	14	0	3	26	10	0	16	27	2	4	21
		(82.3)	(0)	(17.6)		(38.5)	(0)	(61.5)		(7.4)	(14.8)	(77.8)
Total	69	49	0	20	136	34	0	102	102	15	8	79
		(71)	(0)	(29)		(25.7)	(0)	(74.3)		(14.7)	(5.9)	(79.4)
ICPL 85030 x ICPW2	16	3	0	13	43	3	1	39	17	0	0	17
		(18.8)	(0)	(81.3)		(7)	(2.3)	(90.7)		(0)	(0)	(100)
ICPL 85030 x ICPW 6	8	6	0	2	37	0	1	36	-	-	-	-
		(75)	(0)	(25)		(0)	(2.7)	(97.3)	-	-	-	-
ICPL 85030 x ICPW 9	26	8	0	18	63	1	5	57	13	2	1	10
		(30.8)	(0)	(69.2)		(1.6)	(8)	(90.4)		(15.4)	(7.7)	(76.9)
ICPL 85030 x ICPW 12	26	12	0	14	51	5	0	46	20	0	0	20
		(46.2)	(0)	(53.8)		(9.8)	(0)	(90.2)		(0)	(0)	(100)
Total	76	30	0	46	194	9	7	178	50	2	1	47
		(39.5)	(0)	(60.5)		(4.6)	(3.6)	(91.8)		(4)	(2)	(94)

( ) = respective per cent value

Crosses involving ICPL 85030 produced relatively low frequency of male-sterile plants as compared to the crosses with ICP 28. The cross ICP 28 x ICPW 12 yielded 82.3% male-sterile plants in F_1 generation and 38.5% in BC_1F_1 generation, but the BC_2F_1 yielded only 7.4% male-sterile plants.

It was also observed that in all the crosses the frequency of fully fertile plants was less than those of partial fertile. The two crosses (ICP 28 × ICPW 6 and ICP 28 × ICPW 12) which exhibited highest proportion of male-sterility in F_1 generation also segregated in BC_1F_1 for male-sterility and partial fertility in subsequent generations. It was also noted that crosses with ICP 28 had relatively fewer partial fertile plants as compared to those with ICPL 85030. In crosses involving ICP 28, the proportion of partial fertile plants increased gradually from F_1 (29%) to BC_1F_1 (74%) and to BC_2F_1 (79%). Similar observations were recorded in the crosses involving ICPL 85030. The absence of fully fertile plants in most progenies indicated the absence of any dominant fertility restoring genes in the four wild species accessions. Further, the abundance of partial fertile plants with varying degree of pollen fertility suggested polygenic nature of fertility restoration and some minor genes complemented each other to produce fertile genotype. Hence, by each backcross generation, such genes accumulated and resulted in enhanced proportion of fertile plants.

The cytoplasmic male-sterility in the plants is known to occur due to some genetic alterations such as deletion, inversion, or defective transcription of mRNA. The altered mitochondrial DNA assembly adversely affects the normal microsporogenesis in plants that results in partial or complete male-sterility. Elkonin *et al.* (1998) reported that the interaction between nuclear and mitochondrial genomes of distantly related species often induces changes at specific sites in the mitochondrial genome leading to abnormal microsporogenesis. The fertility restoring dominant allele(s) present in the nuclear genome of a restorer line overcomes or repairs the ill effect of inter-genomic interactions and inhibits production of the defective protein/regulatory product, and thereby, permits the plants to produce fertile pollen grains. In a maintainer line of CMS system such repairing/complementing alleles are missing and the progeny of its cross with male-sterile plants will always maintain male-sterility. These processes are not always neat

and some variation with respect to expression of male-sterility and fertility is commonly observed.

Mallikarjuna and Saxena (2005) reported that the F_1 male-sterile segregants when backcrossed to *C. acutifolius* produced all male-sterile plants and suggested that *C. acutifolius* had recessive (*frfr*) alleles that helped in maintaining the male-sterility. Unfortunately, the wild species per se cannot be considered as a standard maintainer because each backcross will enhance the proportion of wild species nuclear genome. Therefore, productive maintainers carrying *frfr* alleles should either be searched among diverse pigeonpea germplasm, or selected from segregating populations of crosses involving *C. acutifolius* and pigeonpea.The genetic materials generated in this study may be vastly different from the existing CMS sources; and hence, concerted efforts are needed to breed good and productive male-sterility maintainers.

The F_1 data recorded in the present study showed that both the pigeonpea genotypes ICP 28 and ICPL 85030 have sterile(S) cytoplasm and the fertility in such genotypes can only be restored by introducing fertility restorer (*Fr*) gene(s) from other genotypes. The number and nature of *Fr* genes, however, may vary among the restorers. According to Kaul (1988), in most of the cases the fertility restoring genes are dominant in nature and their number may vary from one gene to polygenes. However, in some cases partial male-fertility restoration occurs when polygene determines fertility restoration where the genes act together in complementation mode to produce male fertile hybrid plants. Sometimes, polygenes with small contribution by each gene can produce hybrids with full male fertility (Kaul, 1988). Edwardson and Warmke (1967) observed that in petunia (*Petunia hybrida*) pollen production was induced by interactions between non-restorer genes, modifying genes, and certain environmental factors. In the present case all the *C. acutifolius* accessions lacked the major dominant *Fr* gene; but the subsequent backcross data suggested the presence of some minor genes that controlled the fertility restoration. This resulted in the accumulation of such genes with each backcross and thereby enhances the male fertility.

To make use of this sterile (S) cytoplasm of pigeonpea, besides selecting the maintainers, a few fertility restorers also need to be bred or searched among the germplasm. Considering the importance of the cytoplasmic diversity in CMS-based hybrid breeding programs, the successful development of A/B lines and their restorers will have lasting implications in breeding diverse pigeonpea hybrid cultivars.This may ensure greater production stability in the wake of emergence of new biotic and abiotic stresses induced by climate change.

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REFERENCES

Dundas IS, Britten EJ, Byth DE and Gordon GH. 1986. Australian *Atylosia* species –a new gene source for pigeonpea breeders. In: Proceedings of the Fifth International congress (SABRAO). Napompeth B and Subhadrabandhu S (Eds.) Pp. 389-395.

Dundas IS. 1990. Pigeonpea: Cytology and cytogenetics-perspectives and prospects. The pigeonpea (Nene YL, Hall, SD and Sheila VK Eds.). Pp. 117-136.

Edwardson JR and Warmke HE. 1967. Fertility restoration in cytoplasmic male-sterile petunia. Journal of Heredity **58**(4): 195-196.

Elkonin LA, Kozhemyakin VV and Ishin AG. 1998. Nuclear-cytoplasmic interactions in restoring of male fertility in the '9E' and A_4 CMS-inducing cytoplasms of sorghum. Theoretical and Applied Genetics **97**: 626-632.

Joginder S, Bajpai GC and Tewari SK. 2004. Cytogenetic analysis of interspecific hybrids in genus *Cajanus*. Indian Journal of Pulses Research**17**: 14-16.

Kaul MLH. 1988. Male sterility in higher plants. In: Frankel R, Grossman M, Maliga P, Riley R, eds. Monographs of Theoretical & Applied Genetics 10. Heidelberg: Springer-Verlag, pp.775–797.

Mallikarjuna N and Kalpana N. 2004. Mechanism of CMS in pigeonpea wide cross *Cajanus cajan* x *C.acutifolius*. Indian Journal of Genetics and Plant Breeding **64**: 115-117.

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

Ohri D and Singh SP. 2002. Karyotypic and genome size variation in *Cajanus cajan* (L.) Millsp. (Pigeonpea) and some wild relatives. Genetic Resources and Crop Evolution **49**: 1-10.

Ratnaswamy R,Yolanda LJ, Kalaimagal T, Suryakumar M, and Sasikumar D. 1999. Cytoplasmic-genic male sterility in pigeonpea. Indian Journal Agricultural Sciences **69**:158-160.

Saxena KB and Nadarajan N. 2010. Prospects of pigeonpea hybrids in Indian agriculture. Electronic Journal of Plant Breeding **1**:1107-1117.

Saxena KB, Sultana R, Mallikarjuna N, Saxena RK, Kumar RV, Sawargaonkar SL and Varshney RK. 2010. Male-sterility systems in pigeonpea and their role in enhancing the yield. Plant Breeding **129**: 125-134.

van der Maesen LJG.1986. *Cajanus* DC and *Atylosia* W & A.(Leguminosae). Agricultural University, Wageningen,(Agricultural University Wageningen papers).Pp 225.

Xu GW, Cui YY, SchertzKF and Hart GE. 1995. Isolation of mitochondrial DNA sequences that distinguish male-sterility influencing cytoplasm in *Sorghum bicolor* (L.) Moench. Theoretical and Applied Genetics **90**: 1180-1187.