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Inheritance of fertility restoration in pigeonpea

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ABSTRACT

Fertility restoration system in five CMS-based pigeonpea [(*Cajanus cajan* (L.) Millspaugh] hybrids was studied during *kharif* 2010 at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh. Two hybrids 'ICPH 2671' and 'ICPH 2740' which had the same male parent but different females segregated in F_2 in the ratio of 12 fertile (F) : 3 partial fertile (PF) :1 sterile (S), and in BC_1F_1 generation as 2 fertile : 1 partial fertile : 1 sterile, suggesting that fertility restoration in these hybrids was controlled by digenic dominant epistatic interaction. The progenies derived from hybrid 'ICPH 3359' fitted well to an F_2 ratio of 9 F : 6 PF : 1 S, and 1 F : 2 PF : 1 S in BC_1F_1 generation, indicating the involvement of two major genes with incomplete dominant epistasis. Progenies of the other two hybrids 'ICPH 4012' and 'ICPH 4344' segregated in F_2 in the ratio of 9 F : 3 PF : 4 S, and 1 F : 1 PF : 2 S in BC_1F_1 generations, suggesting that pollen fertility was controlled by digenic recessive epistatic gene action. Results of the present investigation revealed that fertility restoration of A_4 CMS system in pigeonpea was governed by two major genes but with different types of epistatic interactions in different crosses.

***Key words*:** Dominant gene, Epistasis, Fertility restoration, Hybrid, Pigeonpea

Pigeonpea [*Cajanus cajan* (L.) Millspaugh] is a short-lived perennial member of family *Fabaceae* and is invariably cultivated as annual crop. It is an often cross-pollinated (20-70%) crop with 2n = 2x = 22 chromosomes. Globally, pigeonpea is grown on 4.5 million hectares land in more than 20 countries with an annual production of 3.48 million tons (FAO 2011). Since 1976, pigeonpea has globally recorded a 56% increase in its area and production but the productivity has remained low at 700 kg/ha (http://faostat.fao.org/site/339/default.aspx). Progress through genetic improvement of yield potential has been limited, and the improved cultivars developed through breeding could not enhance the productivity of the crop in the last five decades (Singh *et al*. 2005). Also, the genetic male sterility (GMS) based pigeonpea hybrids could not be commercialized because of high seed cost and difficulties in maintaining the genetic purity (Saxena *et al*. 2006, Saxena and Nadarajan 2010). Hence, the development of cytoplasmic nuclear male sterility (CMS) became imperative.

Cytoplasmic nuclear male sterility (CMS) is a maternally inherited trait and does not follow Mendelian laws of segregation; and this can originate from alternations in either nuclear or cytoplasmic genes. CMS has been reported in about 140 plant species belonging to 47 genera and 20 families (Kaul 1988). In this system the genetic determinants of male sterility generally inherit through the mitochondrial genome. However, the nuclear genomes also play an important role in the expression of CMS phenotype (Newton 1988). CMS has been conveniently used in hybrid breeding programme in a number of crop species since it eliminates the expensive hand emasculation procedures. In pigeonpea, seven CMS systems were developed by integrating the cytoplasm of wild species with the genome of cultivars through interspecific hybridization followed by selection and backcrossing (Saxena *et al*. 2010a). Of these, A_4 CMS system derived from a cross involving a wild relative of pigeonpea (*C. cajanifolius*) and cultivated type (*C. cajan*) has shown great promise (Saxena *et al*. 2005) because of its stable expression under various agro-climatic conditions, availability of reliable maintainers (B-lines), and stable fertility restoration. The presence of greater genetic diversity among fertility restorers enhances the probability of breeding widely adapted high yielding hybrids. The information about the number of genes controlling fertility restoration (*Rf* or *Fr* genes) and their eventual mapping in the pigeonpea genome will facilitate the development of new hybrids and also provide guidance in the introgression of fertility restoring genes in new genetic backgrounds. Therefore, the present study was undertaken to study the genetics of fertility restoration system in pigeonpea using F_1, F_2, and BC_1F_1 generations in five medium maturing pigeonpea hybrid combinations carrying A_4 cytoplasm.

MATERIALS AND METHODS

The genetics of fertility restoration was studied in five single cross hybrids ('ICPH 2671', 'ICPH 2740', 'ICPH 3359', 'ICPH 4012', and 'ICPH 4344') and their corresponding F_2 and test cross (BC_1F_1) progenies. During 2009 *kharif* season, the parental lines were planted at ICRISAT, Patancheru to undertake a crossing programme. The crosses involved four male sterile ('ICPA 2043', 'ICPA 2047', 'ICPA 2092', and 'ICPA 2052') and four known fertility restorer ('ICPL 87119', 'ICPL 20107', 'ICP 10928', and 'MAL-9') lines. To develop test cross progenies, the F_1 hybrids were crossed with their respective CMS lines (Table 1). Simultaneously, the hybrid plants were selfed using muslin cloth bags to produce F_2 seeds. The genetic materials involving F_1s, F_2s, BC_1F_1s, and parents were

planted at ICRISAT, Patancheru, Andhra Pradesh during *kharif* 2010. For each female and male parent, standard check and F_1 hybrids 18 rows were sown; while 54 rows were planted for each F_2 population. Four meter long rows were spaced at 75 cm with plant to plant spacing of 30 cm. A population of 600 - 650 plants was maintained for each F_2 and 200 - 300 for each F_1 hybrid and test cross except that of hybrid 'ICPH 4344' where 131 plants in F_1, 330 in F_2 and 164 in test cross were grown.

Data on pollen fertility/sterility were recorded on each plant of each entry at 50% flowering stage. For this purpose, 10 well developed but closed flower buds were collected randomly from different parts of each plant at anthesis (9 - 10 A.M.). Anthers were extracted from each bud and crushed with a drop of 2% aceto-carmine stain on a micro slide and examined under a light microscope using 100X magnification. Two such microscopic fields were examined for each sample. The round and well stained pollen grains were considered fertile while shrivelled hyaline pollen grains were counted as sterile. Mean of the two microscopic fields was calculated and the proportion of fertile pollens was expressed in percentage. Based on this data, the plants were classified into fertile (>80% pollen fertility), partial fertile (11 - 80% pollen fertility), and sterile (0 - 10% pollen fertility). The goodness of fit to the expected ratios in F_2 and test cross generations was tested using chi-square test.

RESULTS AND DISCUSSIONS

In CMS system, the male sterility trait is never lost or diluted in the succeeding generations of reproduction and their male fertility can be restored by incorporating dominant restorer gene(s). The fertility restoring genes in the nucleus suppress the male sterile phenotype and allow the production of fertile hybrids. These genes are useful when they are dominant since they make the F_1 hybrid plant fertile. Nadarajan *et al*. (2008) reported the extent of incorporation of fertility restoring genes into different cytoplasmic sources *viz*., A_1, A_2 and A_4. They observed that only 11.3% of hybrids restored fertility across the three cytoplasmic sources. Therefore, incorporating the fertility restoring gene(s) into diverse lines and understanding of their inheritance is essential for a dynamic hybrid pigeonpea breeding programme.

Among the five crosses under study, hybrid 'ICPH 2671' and its test cross had high seed setting with an average of 50% success in hand crossing; whereas in the other cross combinations 30 – 38% seed setting (Table 1) was recorded. A total of 1473 F_1 seeds was obtained from 3900 hand pollinations and 1301 BC_1F_1 seeds from 3700 pollinations. All the male parents had >90% pollen fertility. In each hybrid, all the plants were fully fertile indicating that in each case the restorer parent transferred dominant fertility restoring genes to the hybrid. In F_2 generation of 'ICPH 2671', 527 out of 685 plants were fertile, 113 partial fertile and 45 male sterile. This segregation fit well to the expected ratio of 12 F : 3 PF : 1S sterile ($\chi^2 = 2.31$; P = 0.2 - 0.5). In BC_1F_1 generation, 150 out of 289 plants were fertile, 73 plants had partial fertility and 66 plants were male sterile. This fit well to the expected ratio of 2 F : 1 PF : 1 S ratio ($\chi^2 = 0.76$; P = 0.5 – 0.8) suggesting the presence of two fertility restoration loci in the restorer parent; which interact epistatically with masking gene action. The presence of a single dominant allele of the first fertility restoring gene was enough to restore male fertility. The presence of dominant allele at the second loci provided partial fertility restoration but when present together with the other dominant allele in a genotype it resulted in fertility restoration. Similar results were recorded in hybrid 'ICPH 2740' (Table 2), where out of 641 F_2 plants evaluated, 471 were fertile, while 132 plants expressed partial fertility and the rest 38 plants were sterile. This segregation fit well to the expected ratio of 12 F : 3 PF : 1 S ratio ($\chi^2 = 1.36$; P = 0.5). In BC_1F_1 generation, where 241 plants were grown: 110 were male fertile, 69 partial fertile and 62 male sterile. This segregation fit well to the expected ratio of 2 F: 1 PF : 1 S ($\chi^2 = 2.24$; P = 0.2 – 0.5). The segregation for fertility restoration in F_2 and BC_1F_1 of 'ICPH 2671' and 'ICPH 2740' was similar.

Table 1. Descriptions of parental lines used in hybridization for studying genetics of fertility restoration and crossed seeds harvested

Cross	Hybrid	Pedigree of male parent	Pollination	% Success	Seeds harvested
F_1 hybrids					
ICPA 2043 x ICPL 87119	ICPH 2671	C11 x ICP 1-6W3B	600	50	300
ICPA 2047 x ICPL 87119	ICPH 2740	C11 x ICP 1-6W3B	900	35	315
ICPA 2047 x ICPL 20107	ICPH 3359	IPH 487 inbred -2	900	37	333
ICPA 2092 x ICP 10928	ICPH 4012	T-5 (1-4)	900	33	297
ICPA 2052 x MAL-9	ICPH 4344	MAL-9 variety	600	38	228
Total/mean			*3900*	*38.6*	*1473*
Test crosses					
ICPA 2043 x ICPH 2671	-	-	600	48	288
ICPA 2047 x ICPH 2740	-	-	900	34	306
ICPA 2047 x ICPH 3359	-	-	900	33	297
ICPA 2092 x ICPH 4012	-	-	900	30	270
ICPA 2052 x ICPH 4344	-	-	400	35	140
Total/mean			*3700*	*36.0*	*1301*

This was expected since the restorer line used in the development of both the hybrids was the same. Thus, fertility restoration in these two hybrids was due to digenic dominant epistatic interaction.

In F_2 of hybrid 'ICPH 3359', 390 plants were fertile, 226 partial fertile, and 55 male sterile. This segregation fit well to the expected ratio of 9 F : 6 PF : 1 S ($\chi^2 = 7.10$; P = 0.2 – 0.05). In BC_1F_1 generation out of a total of 231 plants, 54 were male fertile, 116 partial fertile and 61 male sterile. This followed a ratio of 1 F : 2 PF : 1 S ($\chi^2 = 0.43$; P = 0.8). This segregation showed that the restorer line 'ICPL 20107' had two loci responsible for the fertility restoration. It was governed by dominant genes with semi-dominance epistatic interaction (Table 2). It was necessary to have dominant alleles at both loci to provide fertility restoration; one dominant allele alone in homozygous or heterozygous condition only provided partial fertility (incomplete dominance). In hybrid 'ICPH 4012' among 626 F_2 plants grown 359 were fertile, 111 partial fertile and 156 sterile. This segregation fit well to the expected ratio of 9 F : 3 PF : 4 S ($\chi^2 = 0.48$; P = 0.5 – 0.8). In BC_1F_1 generation, the population of 212 plants segregated into 55 fertile, 40 partial fertile and 117 sterile and it fit well to the expected ratio of 1 F : 1 PF : 2 S ($\chi^2 = 4.41$; P = 0.8). The presence of homozygous recessive alleles at one locus results in partial fertility, whereas the presence of fertility restoring alleles at the other locus results in male sterility. This segregation confirmed the recessive epistasis gene interaction of pollen fertility in hybrid ICPH 4012' (Table 2). The similar segregation pattern was observed in hybrid 'ICPH 4344' where the F_2 populations segregated in to 182 fertile, 64 partial fertile, and 84 sterile plants and it fit well in a ratio of 9 F : 3 PF : 4 S indicating recessive epistatic gene action ($\chi^2 = 0.17$; P = 0.80 – 0.95). In BC_1F_1 generation, the population segregated in to 36 fertile, 56 partial fertile, and 72 sterile and it followed the expected ratio of 1 F : 1 PF : 2 S ($\chi^2 = 7.32$; P = 0.01). Hybrids 'ICPH 4012' and 'ICPH 4344' had different male and female parents but their segregation patterns for fertility restoration in their F_2s and BC_1F_1s were comparable. This may be attributed to similar genetic constitution of the parents as far as fertility restoration is concerned.

The success in developing hybrids largely depends on the availability of effective fertility restorers and basic understanding of their inheritance. The segregation patterns recorded in this study suggested that the fertility restoration in pigeonpea was governed by two dominant genes with epistatic or incomplete dominant interaction. Hybrids 'ICPH 2671' and 'ICPH 2740' had the same restorer genes and the two male sterile lines segregated in a ratio of 12 F : 3 PF : 1 S in F_2 and 2 F : 1 PF : 1 S in BC_1F_1 generations, confirming their digenic dominance epistatic interaction. Hybrid 'ICPH 3359' showed a segregation ratio of 9 F : 6 PF : 1 S in F_2 and 1 F : 2 PF : 1 S in BC_1F_1 generations indicated the involvement of two epistasis genes with incomplete dominance while 'ICPH 4012' and 'ICPH 4344' segregated in the ratio of 9 F : 3 PF : 4 S and 1 F : 1 PF : 2 S in F_2 and BC_1F_1 generations, respectively. It is to be noted that the male parents of 'ICPH 4012' and 'ICPH 4344' are very diverse in origin but have similar genes for fertility restoration. Hybrid 'ICPH 4012' has the restorer from a line that originated in Australia; while the pollen parent of 'ICPH 4344' comes from Uttar Pradesh in India. Dalvi *et al*. (2008) reported that the fertility restoration in A_4 cytoplasm was governed by the monogenic gene action (3 F : 1 S in F_2; 1 F : 1 S in BC_1F_1), digenic dominance duplicated gene action (15 F : 1 S in F_2; 3 F : 1 S in BC_1F_1), and complementary (9 F : 7 S in F_2; 1 F : 3 S in BC_1F_1) gene action, respectively. The presence of two dominant genes with one basic and one inhibitory gene action in 'ICPL 87119' was reported by Saxena *et al*. (2010b). Saxena *et al*. (2011) also reported the present of both monogenic and digenic inheritance of fertility restoration in extra early maturing hybrids and two duplicate dominance genes in late maturing hybrid with A_4 cytoplasm. Sawargaonkar (2011) also reported the monogenic as well as digenic control of fertility restoration and it was influenced by nuclear background of parental lines. In the present

Table 2. Segregation for fertility restoration in F_1, F_2, BC_1F_1 generations of five crosses

Cross	Generation	No. of plants				Expected ratio	Probability
		Total	Fertile	Partial fertile	Sterile		
ICPA 2043 x ICPL 87119	F_1	201	201	0	0	1:0	-
	F_2	685	527	113	45	12:3:1	0.2 - 0.5
	BC_1F_1	289	150	73	66	2:1:1	0.5 - 0.8
ICPA 2047 x ICPL 87119	F_1	233	233	0	0	1:0	-
	F_2	641	471	132	38	12:3:1	0.5
	BC_1F_1	241	110	69	62	2:1:1	0.2 - 0.5
ICPA 2047 x ICPL 20107	F_1	160	160	0	0	1:0	-
	F_2	671	390	226	55	9:6:1	0.2 - 0.05
	BC_1F_1	231	54	116	61	1:2:1	0.8
ICPA 2092 x ICP 10928	F_1	195	195	0	0	1:0	-
	F_2	626	359	111	156	9:3:4	0.5 - 0.8
	BC_1F_1	212	55	40	117	1:1:2	0.05
ICPA 2052 x MAL-9	F_1	131	131	0	0	1:0	-
	F_2	330	182	64	84	9:3:4	0.8 - 0.95
	BC_1F_1	164	36	56	72	1:1:2	0.01

findings, since all of female parental lines were based on A_4 cytoplasm the differences observed in the inheritance of fertility restoration were attributed to the interaction of genes present in the restorer line and/or a probable variation in the expression of the weaker genes in different genetic backgrounds.

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