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Study of inheritance of fertility restoration in pigeonpea lines derived from *Cajanus cajanifolius*

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Abstract

In the present study, single crosses involving four CMS-lines and three known fertility restorers were studied to determine the genetics of fertility restoration in pigeonpea (*Cajanus cajan* L.

Millsp.). The F₁ plants of all the four hybrids were selfed to produce F₂ seed and simultaneously crossed to their corresponding A-lines to produce BC₁F₁ seeds during 2007. The parents, F₁, F₂, and BC₁F₁ populations were planted in 2008 rainy season at the Marathwada Krishi Vidyapeeth, Parbhani, India. The interaction of dominant nuclear genes of ICP 2766 with ICPA 2092 produced 100% fertile F₁ plant and showed complete dominance for fertility restoration. The restorer ICP 2766 when crossed with ICPA 2092 showed monogenic inheritance (3:1), while ICP 2766 crossed with ICPA 2043 revealed digenic inheritance (15:1) of fertility restoration. In a cross between ICP 2766 and ICPA 2043, the dominant gene of fertility restoration at either of two loci masked the expression of male-sterile recessive alleles at the two loci in such a way that it modified normal di-hybrid ratio in to 15:1 ratio and produced duplicate gene interaction.

Keywords: *Cajanus cajanifolius*-inheritance-male-sterility-male fertility-genetics

Cytoplasmic nuclear-male sterility (CMS) is maternally inherited and associated with specific (mitochondrial) genes without otherwise affecting the plant (Budar and Pelletier 2001). The fertility restorer (*Rf* or *Fr*) genes in the nucleus suppress the male-sterile phenotype and allows commercial exploitation of the CMS system for the production of hybrid seeds. In addition the CMS-*Rf* system provides an excellent model for the study of nuclear-mitochondrial interaction in multicellular organisms. Pigeonpea (*Cajanus cajan* (L.) Millsp.) is the only legume crop where commercial CMS-based hybrids are now available. Two CMS sources are available (were are the coming from A2 cytoplasm (Tikka *et al.* 1997) and A4 cytoplasm (Saxena *et al.*, 2005)) and that the A4 restoration genetics is studied in the present paper. In present study, the F₁ and F₂ generations were developed to determine the nature of gene action in F₁ generation and

segregation pattern in F_2 generation in pigeonpea. In order to take advantage of this CMS hybrid technology, it is essential to breed new high yielding hybrids with diverse genetic backgrounds. To achieve this, breeding of promising hybrid parents and knowledge of the inheritance of fertility restoration are essential.

Materials and Methods

In the present study, single crosses involving four CMS-lines (A4 cytoplasm) and three known fertility restorers (Table 1) were studied to determine the genetics of fertility restoration. The F_1 plants of all the four hybrids were selfed to produce F_2 seeds and simultaneously crossed to their corresponding A-lines to produce BC_1F_1 seeds during 2007. The parents, F_1 , F_2 , and BC_1F_1 populations were planted in 2008 rainy season at Marathwada Krishi Vidyapeeth, Parbhani, India. For each F_2 population, 300 - 500 seeds and for BC_1F_1 population 125 - 175 seeds were sown. To determine the variation for pollen fertility in each generation five fully developed floral buds were collected randomly from each plant and the anthers were squashed in 2% acetocarmine solution. The pollen fertility of each plant was studied in three microscopic fields under light microscope. The fully stained pollen grains were identified as fertile, while the empty or partially stained pollen grains were considered as male-sterile. The goodness of fit to different expected ratios in F_2 and test-cross generations was assessed using a Chi-square test.

Results

The results revealed that all the F_1 plants in the four crosses were male-fertile indicating dominant nature of fertility restoring genes (Table 1). As expected, the F_2 and BC_1F_1 populations of these crosses segregated for male-sterility and fertility. In cross ICPA 2092 x ICP 2766, out of 458 F_2 plants grown, 350 were fertile and 108 were male-sterile. This segregation fit the

expected ratio of 3 fertile: 1 sterile ($p = 0.95$). In BC_1F_1 generation, 64 plants were male-fertile and 59 plants were male-sterile, and it showed a good fit to 1 fertile: 1 sterile ($p = 0.70$) ratio. These observations suggested that the fertility restoration in cross ICPA 2092 x ICP 2766 was controlled by a single dominant gene. Similarly in cross ICPA 2048 x ICP 3477, the F_2 and BC_1F_1 data (Table 1) showed that the fertility restoration was controlled by a single dominant gene.

In cross ICPA 2047 x ICP 3513, the segregation in F_2 generation revealed that out of 424 plants, 393 were male-fertile and only 31 were devoid of pollen grains. This segregation fit the expected di-hybrid ratio of 15 fertile: 1 sterile ($p = 0.87$). In BC_1F_1 generation 110 plants were male-fertile and 43 male-sterile, which showed a good fit for 3 fertile: 1 sterile ($p = 0.63$) ratio. Similarly in cross ICPA 2043 x ICP 2766, out of 315 plants studied, 292 were male-fertile and 23 male-sterile, exhibiting a good fit to a 15 fertile: 1 sterile ($p = 0.87$) ratio. In $BC_1 F_1$ generation out of 111 plants grown, 84 were male-fertile and 27 were male-sterile. This segregation fit the expected ratio of 3 fertile: 1 sterile ($p = 0.57$) ratio. The segregation patterns observed in these two crosses suggested the presence of two duplicate dominant genes in controlling the pollen fertility of CMS lines ICPA 2047 and ICPA 2043.

Discussion

The genetics of fertility restoration is important for the transfer of restorer genes from one genotype to another. In the present study, it was observed that the restorer ICP 2766 when crossed with ICPA 2092 showed monogenic inheritance (3:1), while the cross with another ICPA 2043 revealed digenic inheritance of fertility restoration. Dalvi *et al.* (2008) studied the inheritance of fertility restoration in three diverse early maturing CMS lines of pigeonpea and

reported that fertility restoration in three pigeonpea crosses was controlled by one dominant gene; while in two crosses two duplicate dominant genes controlled fertility restoration. Nadarajan *et al.* (2008) reported variable restoration patterns among a common set of male parents within a single cytoplasmic source of pigeonpea. In the present study fertility restorer line ICP 2766, when crossed to two different A-lines produced different results as far as their fertility restoration was concerned. In cross ICPA 2092 x ICP 2766, the fertility restoration was controlled by a single dominant gene, while in cross ICPA 2043 x ICP 2766 a di-genic duplicate dominance inheritance of fertility restoration was observed. The differential expression of fertility restoring genes of ICP 2766 can be attributed to different genetic backgrounds of the F₁ plants, arising from female parents of different genetic constitution. Such a variable restoration among cytoplasmic sources with the same set of male parents in pigeonpea had been reported (Saxena and Kumar, 2003; Saxena *et al.* 2005). Saxena *et al.* (2010) revealed that the fertility restoration in A₄ CMS lines of pigeonpea was controlled by either one or two fertility restoring genes. Bai and Gai (2005) observed that in soybean fertility restoration was controlled by monogenic to digenic gene in CMS lines of NJCMS2A. Mono-factorial inheritance associated with fertility restoration in red pepper (*Capsicum annum* L.) was reported by Gulyas *et al.* (2006). Schertz (1994) reported that fertility restoration in sorghum (*Sorghum bicolor* (L.) Moench) was dependent on interaction of cytoplasm and nuclear genes and was controlled by a single dominant gene in some combinations but by two or more dominant genes in other combinations. This happened when the same nuclear genes interacted with different cytoplasm. The differential reactions of CMS-lines with the same cytoplasm in test crosses may be caused by the action of recessive nuclear genes of the recurrent male parents that were accumulated during backcrossing which may induce changes in cytoplasmic genes controlling male-sterility

(Elkonin *et al.* 1998). Differences observed in segregation patterns also could be due to the presence of some modifier genes that influence the process of penetrance and expressivity of the fertility restoring genes (Hossain *et al.* 2010).

In the present study the inheritance of male-fertility restoration in CMS-lines of pigeonpea was cross-specific and influenced by the nuclear background of male-sterile and fertility restoring lines. In crosses ICPA 2048 x ICP 3477 and ICPA 2092 x ICP 2766, it was controlled by a single dominant gene; while in crosses ICPA 2043 x ICP 2766 and ICPA 2047 x ICP 3513 the fertility restoration was determined by two duplicate dominant genes. The differential behavior of one fertility restorer line (ICP 2766) in two different crosses was attributed to the interactions of different nuclear genes of the two female parents.

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Table 1: Segregation ratios for male-fertile and sterile plants in F₁, F₂ and BC₁F₁ generations of crosses involving A₄ CMS-lines and fertility restorers in pigeonpea

Cross	Generat ion	Number of plants			Ratio	Chi-square probability
		Total	Fertile	Sterile		
ICPA 2092 x ICP 2766	F ₁	5	5	0	-	-
	F ₂	458	350	108	3:1	0.95
	BC ₁ F ₁	123	64	59	1:1	0.7
ICPA 2043 x ICP 2766	F ₁	7	7	0	-	-
	F ₂	315	292	23	15:1	0.87
	BC ₁ F ₁	111	84	27	3:1	0.57
ICPA 2048 x ICP 3477	F ₁	9	9	0	-	-
	F ₂	417	318	99	3:1	0.96
	BC ₁ F ₁	114	53	61	1:1	0.51
ICPA 2047 x ICP 3513	F ₁	17	17	0	-	-
	F ₂	424	393	31	15:1	0.87
	BC ₁ F ₁	153	110	43	3:1	0.63