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GENETICS AND PLANT BREEDING

Genetics of fertility restoration and agronomic performance of CMS based hybrids in pigeonpea

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Abstract

In the study, CMS based hybrid combination involving two male sterile lines (*Cajanus cajanifolius*) and four restorer lines in pigeonpea (*Cajanus cajan)* were studied to determine the genetics of fertility restoration in pigeonpea (*Cajanus cajan* L. Millsp.). Based on the pollen fertility and pod setting, F_2 segregating populations were categorised. Out of two major genes governing the fertility restoration, one gene segregated in the ratio of 9:3:4 whereas the second gene in 12:3:1 due to the allelic differences. The ICPA 2043/ LRG 41 and ICPA 2043/NDA 1 hybrids fits best for agronomical aspects as they showed earliness with respect to flowering and maturity having more number of pods plant^{-1}, increase in pod length along with more seed yield plant^{-1}. CMS system is a useful tool serving to increase seed yield production of various crops and consummate the demands of increasing populations.

Highlights

- Fertility restoration was observed to be governed by two major genes.
- The seed yield of hybrids was extremely high i.e. 62.12 and 12.18 and 67.32 and 15.78 per cent over the checks LRG 41 and NDA1 respectively.

Keywords: Epistasis, restorer, pollen fertility, hybrid, pigeonpea

Pigeonpea [*Cajanus cajan* (L.) Millspaugh], a short lived perennial member of family *Fabaceae*, 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 ~6.22 m ha land in more than 20 countries with an annual production of ~4.74 MT (FAOSTAT 2015).

Since 1976, pigeonpea has recorded globally a 56% increase in its area and production but the productivity has remained low at ~750kg/ha (FAOSTAT 2015). Singh *et al.* (2005) reported that the progress through genetic improvement of yield potential is limited and the improved cultivars developed through breeding has been inadequate in enhancing the productivity of the crop in the last five decades. Moreover, the genetic male sterility (GMS) based pigeonpea hybrids has not been 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 do not follow Mendelian law of segregation; and this can originate from alternations in either nuclear or cytoplasmic genes. 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 reported in about 140 plant species belonging to 47 genera and 20 families (Kaul 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 (A_1, A_2, A_3, A_4, A_5, A_6 and A_7, A_8) 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.* 2013). Among 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 regarding 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. Consequently, this will provide guidance in the introgression of fertility restoring genes in new genetic backgrounds. Keeping this in view, 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 16 long maturing pigeonpea hybrid combinations carrying A_4 cytoplasm.

Materials and methods

Two CMS (flat, translucent anthers with a whitish scaly surface) and four restorer (pollinator) lines were used in the study (Table 1). The CMS lines ICPA 2043 and ICPA ICP 2092A inherited A_4 cytoplasm imparting male sterility. Restorer lines ICP 3760, ICPR 3802, LRG 41 and NDA 1 were crossed in using line / tester fashion. Pollen fertility was tested for all the F_1 plants at two temperatures i.e. at low (~27°C during November/ December) and high (~33°C during March) temperature in *Kharif* 2009-10, 2010-11 and 2011-12. Stable F_1 hybrids exhibiting more than 90% fertility (in *Kharif* 2009-10, 2010-11) were selected and used as pollinator to make cross with their respective CMS lines for developing test crosses progenies in *Kharif* 2010-11. The genetic materials involving F_1s, F_2s, BC_1F_1s, and parents were planted at Agricultural research farm, Banaras Hindu University Varanasi, India during *Kharif 2011*-12. Parents and F_1 hybrids were sown 3 rows each while each F_2 and test cross populations were sown 15 and 6 rows respectively. Each plot consisted of a single row of four meter length with inter and intra row spacing of 75cm and 25 cm, respectively. A population of 76-96, 48, 198 – 240 and plants was maintained for each test cross, F_1 and F_2 and generation respectively.

Pollen scoring

Data on pollen fertility/sterility were recorded on each plant of each entry at 50% flowering stage. To determine the variation in pollen fertility in each generation, three fully developed floral buds were collected randomly from each plant and the anthers were squashed in 2% aceto-carmine stain on a micro slide and examined under a light microscope using 100x magnification. Five microscopic fields for each sample were examined. Mean of the five microscopic fields was calculated and the proportion of fertile pollens was expressed in percentage. Based on percent pollen fertility, the plants were classified as fertile (90% and more), partially fertile (11-90%) and sterile (10% and less) (Fig. 1). The goodness of fit to Mendelian segregation behaviour of fertile, partially fertile and sterile plants in F_2 and test cross generations was tested by chi-square (χ^2) technique.

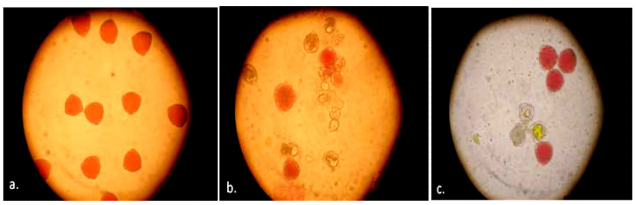

Fig. 1: Pollen Fertility (a) Fertile pollen; (b) Sterile pollen; (c) Partially fertile pollen

Results and discussion

CMS system is a maternally inherited trait governed by specific (mitochondrial) genes which have no any significant effect on other properties of the plant (Budar and Pelletier, 2001). The genes for fertility restorer (Rf or Fr) genes prevailing in the nucleus suppresses the expression of male-sterile phenotype, consequently leading to commercial exploitation of the CMS system for the production of hybrid seeds. Commercially exploitable CMS system has not been found in cultivated pigeonpea. In this context, various wild relatives have been utilized to develop CMS system. It has been observed that the CMS system containing A_2 cytoplasm has resulted in reduced reproductive fitness of plants. This may be due to presence of several undesirable wild genes from *C. scarabaeoides*. On the other hand, *C. cajanifolius*, which is the immediate progenitor

Table 1: CMS and restorer lines used in the study

Sl. No.	CMS/Restorer lines	Parentage	Origin/ source	Morphological characters
	Lines			
1	ICPA 2043	*Cajanus cajanifolius*	ICRISAT, Hyderabad	Compact plant type, medium plant height, yellow flower, pod green, brown seed colour
2	ICPA 2092	-do-	-do-	Spreading plant type, medium plant height, yellow flower, pod green with stripes, reddish brown seed colour
	Testers			
3	ICPR 3760	*Cajanus cajan*	-do-	Compact plant type, semi-compact, Pod green with streaks, Yellow flower, gray seed with red streaks
4	ICPR 3802	-do-	-do-	Semi-spreading, Pod green with streaks, Yellow flower, reddish brown seed
5	LRG 41	-do-	Andhra Pradesh	Semi spreading plant type, stem green, pod green with streaks, flower yellow with light reddish streaks, brown mature pod colour, light brown seed colour
6	NDA 1	-do-	Selection of Faizabad	Semi- Compact, indeterminate, pod green, yellow flower with reddish streaks, brown mature pod colour, brown seed colour

of pigeonpea, resembles cultivated types in most morphological and agronomic traits (De, 1974). The male-sterile lines derived from A_4 cytoplasm of *C. cajanifolius* have appeared to be the best as they do not display any morphological deformity or reduction in reproductive fitness (Dalvi *et al.* 2008a). Thus, for the transfer of restorer genes from one genotype to another the acquaintance of inheritance pattern of fertility restoration is indispensable.

In the present study, data for pollen fertility of every test genotypes, F_1, F_2 and BC_1F_1 populations recorded at two temperatures, low (~27°C during November/ December) as well as high (~33°C during March) to observe the effect of temperature (thermo-sensitivity) on pollen fertility, over three crop season i.e. 2009-10, 2010-11 and 2011-12 were recorded which is depicted in Table 2.

The F_1 hybrids and their parents were tested with check varieties (LRG 41 and NDA 1) for the yield components, viz. days to 50% flowering, days to maturity, number of pods plant^{-1}, pod length and seed yield plant^{-1} (Table 3). On the basis of recorded data, the days to 50% flowering was low i.e. 103.39 for ICPA 2043 / LRG 41 and 103.42 for ICPA 2043 / NDA, medium i.e. 104.60, 104.33 and 104.00 for ICPA 2092 / LRG 41, ICPA 2092 / ICPR 3760 and ICPA 2043 / ICPR 3802 crosses respectively, whereas it was high for ICPA 2092 / NDA 1, ICPA 2092 / ICPR 3802 and ICPA 2043 / ICPR 3760 having mean 109.82, 106.71 and 106.53 days respectively. Days to maturity ranged between 228.48 (ICPA 2043 / ICPR 3760) and 247.08 (ICPA 2092 / ICPR 3802). Similarly, number of pods plant^{-1}, was found to be very high (238.72) in ICPA 2043 / LRG 41 and low (137.29) in ICPA 2092 / ICPR 3802 cross. The pod length was low (3.19) in ICPA 2043 / ICPR 3802 and high (5.11) in ICPA 2092 / ICPR 3760 cross. The seed yield plant^{-1} of the hybrids was found to be high in ICPA 2043 / NDA 1(41.11g) and ICPA 2043 / LRG 41 (39.83g) which were 67.32 % and 62.12 % and 15.78% and 12.18% higher than both the checks LRG 41 and NDA 1, respectively. Among all the eight hybrids, hybrid ICPA 2043 / NDA 1 and ICPA 2043 / LRG 41 showed better performance over both checks LRG 41 and NDA 1,with respect to traits like seed yield, flowering, maturity and number of pods plant^{-1} and pod length. This can prove to be an enormous achievement for pigeonpea under late maturing category.

The pigeonpea breeding started in the early 1960's. The first pigeonpea hybrid with the hybrid vigour was reported in mid 20th century by Solomon *et al.* (1957). Later on, several reports on hybrid vigor for yield and yield component traits in pigeonpea, were published (Saxena and Sawargaonkar 2014). Still, during this time CMS was not reported and GMS-based hybrids ruled having heterosis for yield upto 35–60% heterosis (Saxena *et al.* 2005). After the introduction and utilization of CMS in hybrid breeding programme, standard heterosis showed a boost up to 156% for grain yield and gained yield advantage of 50–100% (Saxena 2007). In the current

Table 2: Mean of the pollen fertility of parents and F_1 hybrids

Family	Pollen fertility					
	2009-2010		2010-2011		2011-2012	
	Low temp. (~28°C)	High temp. (~33°C)	Low temp. (~27 °C)	High temp. (~34°C)	Low temp. (~27°C)	High temp (~33°C)
Lines						
ICPA 2043	0.00	0.00	0.00	0.00	0.00	0.00
ICPA 2092	0.00	0.00	0.00	0.00	0.00	0.00
Testers						
ICPR 3760	100	100	100	100	100	100
ICPR 3802	100	100	100	100	100	100
LRG 41	100	100	100	100	100	100
NDA 1	100	100	100	100	100	100
Hybrid						
ICPA 2043 / ICPR 3760	94.95	97.09	96.22	97.23	97.23	97.82
ICPA 2092 / ICPR 3760	97.09	95.62	96.87	97.56	96.94	97.62
ICPA 2043 / ICPR 3802	94.35	97.42	95.87	96.41	96.38	97.43
ICPA 2092 / ICPR 3802	94.39	94.68	96.74	97.74	97.38	97.72
ICPA 2043 / LRG 41	94.63	96.26	95.24	96.24	96.30	96.39
ICPA 2092 / LRG 41	97.09	95.23	94.87	95.53	95.28	97.28
ICPA 2043 / NDA 1	96.92	98.04	93.29	97.50	98.04	98.44
ICPA 2092 / NDA 1	96.61	96.33	94.71	95.38	96.19	97.03

study, two F_1 hybrids, viz. ICPA 2043 / NDA 1 and ICPA 2043 / LRG 41, performed best out of eight for yield-related traits over both the check varieties (LRG 41 and NDA 1). Of the eight crosses, two hybrids ICPA 2043 / NDA 1 and ICPA 2043 / LRG 41showed better yield, 67.32% and 15.78 and 62.12 % and 12.18% higher yield advantage over both the checks. The CMS-based hybrid ICPH 2740 and ICPH 3762 recently released from ICRITSAT have proved the advantage of CMS-based hybrids with the 25–30% yield advantage over the other hybrids (Saxena and Sawargaonkar 2014). Similarly, Saroj *et al.* (2015) reported ICPA 2043 / Azad, CMS-based long duration pigeonpea hybrid from BHU have showed 29.79% and 53.74% higher yield advantage over both the best local checks MAL 13 and NDA 1.

In the present investigation, the results among the eight individual crosses exhibited that in hybrid ICPA 2043 / ICPR 3760, out of 221 plants 139 were fertile, 31 partially fertile and rest 51 male sterile. This segregation fits well into a ratio of 9 F: 3PF: 4S (χ^2= 4.69; P = 0.10) (Table 4). Similarly, BC_1F_1 generation, revealed a good fit for a 1 F: 1PF: 2S ratio (χ^2= 5.08; P = 0.08). This revealed the involvement of digenic supplementary or an epistasis with recessive gene action. Assuming that Rf_1 and Rf_2 were the dominant alleles of the two restorer genes, the fertility restoring action of Rf_1 seemed to be stronger than Rf_2 (Table 5). The segregation behaviour in the cross combination indicated that when both dominant genes were present together in heterozygous or homozygous condition (Rf_1-Rf_2-) the plants were fully fertile. The homozygous rf_2rf_2 plant with homozygous dominant Rf_1Rf_1 or heterozygous Rf_1- rf_2 for Rf_1 gene falls in the partial fertile group. The homozygous rf_1rf_1 plant with homozygous dominant Rf_2Rf_2 or heterozygous dominant Rf_2rf_2 for Rf_2 locus were completely sterile. The dominant allele of Rf_2 gene did not show any effect of fertility restoration in the absence of other dominant allele of the Rf_1 locus. Thus, two loci appeared to have additive effects in imparting full fertility restoration. The plants homozygous for recessive alleles of both the genes rf_1rf_1 rf_2rf_2 were completely sterile. In BC_1F_1 generation, out of 78 plants, 12 were fertile, 18 partial fertile and 48 had male sterile anthers and which revealed 1 F: 1PF: 2S ratio (χ^2= 5.08; P = 0.01). This segregation confirmed the recessive epistasis (supplementary) gene action of pollen fertility in this hybrid. The similar result

Table 3: Performance of F_1 hybrids and their parents for important agronomic traits in pigeonpea

S. No.	Hybrids	Days to 50% flowering	Days to maturity	Number of Pods Plant^{-1}	Pod Length	Seed yield Plant^{-1} (g)	Yield (Kg/ha)	LRG 41	Yield (%) with LRG 41	NDA 1	Yield (%) with NDA 1
1	ICPA 2043 / ICPR 3760	106.53	228.48	166.95	3.46	29.52	1574.13	263.75	20.13	-319.64	-16.88
2	ICPA 2092 / ICPR 3760	104.33	233.37	147.48	5.11	31.16	1661.87	351.48	26.82	-231.91	-12.25
3	ICPA 2043 / ICPR 3802	104.00	234.04	175.23	3.19	33.52	1787.91	477.53	36.44	-105.87	-5.59
4	ICPA 2092 / ICPR 3802	106.71	247.08	137.29	3.98	27.64	1474.07	163.69	12.49	-419.70	-22.16
5	ICPA 2043 / LRG 41	103.39	233.92	238.72	4.58	39.83	2124.39	814.00	62.12	230.61	12.18
6	ICPA 2092 / LRG 41	104.60	236.12	199.49	4.99	32.22	1718.22	407.84	31.12	-175.56	-9.27
7	ICPA 2043 / NDA 1	103.42	230.42	216.08	4.38	41.11	2192.53	882.15	67.32	298.76	15.78
8	ICPA 2092 / NDA 1	109.82	238.18	166.89	4.64	30.88	1647.17	336.79	25.70	-246.61	-13.02
	Mean	105.35	235.20	181.02	4.29	33.24					
	Variance	4.88	32.16	1195.40	0.48	23.09					
	Sem (±)	0.78	2.00	12.22	0.25	1.70					
	CV (%)	2.10	2.41	19.10	16.20	14.46					
	Lines										
9	ICPA 2043	106.02									
10	ICPA 2092	104.33									
	Mean	105.18									
	Variance	1.43									
	Sem (±)	0.845									
	CV (%)	1.14									
	Maintainers										
11	ICPB 2043	108.41	237.41	165.62	4.51	35.7					
12	ICPB 2092	108.67	235.78	177.69	4.53	33.43					
	Mean	108.54	236.60	171.66	4.52	34.57					
	Variance	0.03	1.33	72.84	0.00	2.58					
	Sem (±)	0.13	0.82	6.03	0.01	1.14					
	CV (%)	0.17	0.49	4.97	0.40	4.64					
	Testers										
13	ICPR 3760	111.00	234.43	144.49	3.40	28.21					
14	ICPR 3802	115.00	238.67	156.40	4.36	31.21					
15	LRG 41	111.33	244.33	120.50	4.56	24.57					
16	NDA 1	110.41	236.10	183.85	5.33	35.51					
	Mean	111.94	238.38	151.31	4.41	29.87					
	Variance	4.32	18.77	693.51	0.63	21.48					
	Sem (±)	1.04	2.17	13.17	0.40	2.32					
	CV (%)	1.86	1.82	17.40	18.01	15.52					
	Checks										
17	LRG 41	111.33	244.33	120.50	4.56	24.57	1310.39				
18	NDA 1	110.41	236.10	183.85	5.33	35.51	1893.78				

was found in these hybrids ICPA 2092 / ICPR 3802. The results were found to be in corroboration with the earlier findings in pigeonpea (Kyu and Saxena, 2011; Saroj *et al.* 2015).

The inheritance study of pollen fertility restoration in F_2 generation of 'ICPA 2092 / ICPR 3760', 162 out of 231 plants were fertile, 47 partial fertile and 22 male sterile. This segregation fit well to the expected ratio of 12 F: 3 PF: 1S sterile ($\chi^2 = 5.01$; P = 0.08). In BC_1F_1 generation, 49 out of 84 plants were fertile, 12 plants had partial fertility and 23 plants were male sterile. This fit well to the expected ratio of 2

Table 4: Pollen fertility of plants in F_2 population obtained from the crosses of CMS lines with restorer lines

Crosses	Generation	No of Plants				Expected ratio	χ^2 value	Probability
		Total	Fertile	Partial fertile	Sterile			
ICPA 2043 / ICPR 3760	F_1	48	48	0	0	1:0		
	F_2	221	139	31	51	9:3:4	4.69	0.10
	BC_1F_1	78	12	18	48	1:1:2	5.08	0.08
ICPA 2092 / ICPR 3760	F_1	48	48	0	0	1:0		
	F_2	231	162	47	22	12:3:1	5.01	0.08
	BC_1F_1	84	49	12	23	2:1:1	5.21	0.07
ICPA 2043 / ICPR 3802	F_1	48	48	0	0	1:0		
	F_2	234	169	42	23	12:3:1	5.12	0.08
	BC_1F_1	96	53	25	18	2:1:1	2.06	0.36
ICPA 2092 /ICPR 3802	F_1	48	48	0	0	1:0		
	F_2	240	149	36	55	9:3:4	3.67	0.16
	BC_1F_1	94	19	23	52	1:1:2	1.40	0.50
ICPA 2043 / LRG 41	F_1	48	48	0	0	1:0		
	F_2	210	147	42	21	12:3:1	5.60	0.06
	BC_1F_1	81	51	13	17	2:1:1	5.84	0.05
ICPA 2092 / LRG 41	F_1	35	35	0	0	1:0		
	F_2	198	151	28	19	12:3:1	5.83	0.05
	BC_1F_1	80	43	25	12	2:1:1	4.68	0.10
ICPA 2043 / NDA 1	F_1	48	48	0	0	1:0		
	F_2	211	171	28	12	12:3:1	4.51	0.11
	BC_1F_1	76	43	21	12	2:1:1	3.45	0.18
ICPA 2092 / NDA 1	F_1	37	37	0	0	1:0		
	F_2	198	161	26	11	12:3:1	4.54	0.10
	BC_1F_1	86	53	18	15	2:1:1	4.86	0.09

F: 1 PF: 1 S ratio (χ^2 = 5.21; P = 0.07) indicating the involvement of two dominant epistatic genes (χ^2 = 5.21; P = 0.07) (Table 4 and 5). Assuming that two dominant gene Rf_1 and Rf_2 seems to control the fertility restoration. The effect of one of the two dominant genes (Rf_1) in restoring fertility appears to be strong and as good as the two together while the other gene (Rf_2) revealed weak restoration. The homozygous or heterozygous plants for both the dominant genes (Rf_1 -Rf_2 -) and those having homozygous or heterozygous dominant gene (Rf_1 -) and homozygous recessive gene (rf_2rf_2) were fully fertile. This indicated that the strong dominant gene Rf_1 is able to control the fertility restoration. While the plants homozygous for rf_1rf_1 and homozygous dominant (Rf_2Rf_2) or heterozygous dominant (Rf_2rf_2) at Rf_2 locus were partial fertile. The plants homozygous for recessive alleles of both the genes (rf_1rf_1 rf_2rf_2) were completely sterile. Similarly, the F_2 and testcross progenies of the crosses ICPA 2043 / ICPR 3802, ICPA 2043 / LRG 41, ICPA 2092 / LRG 41, ICPA 2043 / NDA 1 and ICPA 2092 / NDA 1 exhibited a similar trend of segregation ratio of 12F: 3PF: 1S and 2F: 1PF: 1S, respectively. The ratios confirmed that dominant epistatic gene action is present and both the dominant genes are responsible for controlling the fertility restoration. Similar findings have been reported by earlier workers (Kyu and Saxena, 2011; Saroj *et al.* 2015).

Nadarajan *et al.* (2008) reported that a variable restoration patterns are existing among a common set of restorer lines (male parents) within a single cytoplasmic source of pigeonpea. Dalvi *et al.* (2008b) reported that the fertility restoration in A_4 cytoplasm is governed by the monogenic gene action, digenic dominance duplicate gene action, as well as complementary gene action. Sawargaonkar (2011) also reported that the monogenic as well as digenic gene action controls the fertility restoration

Table 5: Proposed genetic constitution of CMS lines, restorers lines and their F_2 segregants

S. No.	Cross combination	CMS	Restorers	Genetic constitution		
				F_2 segregation		
				F	PF	S
1.	ICPA 2043 / ICPR 3760	$rf_1rf_1\ rf_2rf_2$	$Rf_1Rf_1\ Rf_2Rf_2$	9 Rf_1-Rf_2 -	3 Rf_1- rf_2rf_2-	3 $rf_1rf_1\ Rf_2$ – 1 $rf_1rf_1\ rf_2rf_2$
2.	ICPA 2092 / ICPR 3760	$rf_1rf_1\ rf_2rf_2$	$Rf_1Rf_1\ Rf_2Rf_2$	9 Rf_1-Rf_2 - 3 Rf_1- rf_2rf_2	3 $rf_1rf_1\ Rf_2$ -	1 $rf_1rf_1\ rf_2rf_2$
3.	ICPA 2043 / ICPR 3802	$rf_1rf_1\ rf_2rf_2$	$Rf_1Rf_1\ Rf_2Rf_2$	9 Rf_1-Rf_2 - 3 Rf_1- rf_2rf_2	3 $rf_1rf_1\ Rf_2$ -	1 $rf_1rf_1\ rf_2rf_2$
4.	ICPA 2092 /ICPR 3802	$rf_1rf_1\ rf_2rf_2$	$Rf_1Rf_1\ Rf_2Rf_2$	9 Rf_1-Rf_2 -	3 Rf_1- rf_2rf_2-	3 $rf_1rf_1\ Rf_2$ – 1 $rf_1rf_1\ rf_2rf_2$
5.	ICPA 2043 / LRG 41	$rf_1rf_1\ rf_2rf_2$	$Rf_1Rf_1\ Rf_2Rf_2$	9 Rf_1-Rf_2 - 3 Rf_1- rf_2rf_2	3 $rf_1rf_1\ Rf_2$ -	1 $rf_1rf_1\ rf_2rf_2$
6.	ICPA 2092 / LRG 41	$rf_1rf_1\ rf_2rf_2$	$Rf_1Rf_1\ Rf_2Rf_2$	9 Rf_1-Rf_2 - 3 Rf_1- rf_2rf_2	3 $rf_1rf_1\ Rf_2$ -	1 $rf_1rf_1\ rf_2rf_2$
7.	ICPA 2043 / NDA 1	$rf_1rf_1\ rf_2rf_2$	$Rf_1Rf_1\ Rf_2Rf_2$	9 Rf_1-Rf_2 - 3 Rf_1- rf_2rf_2	3 $rf_1rf_1\ Rf_2$ -	1 $rf_1rf_1\ rf_2rf_2$
8.	ICPA 2092 / NDA 1	$rf_1rf_1\ rf_2rf_2$	$Rf_1Rf_1\ Rf_2Rf_2$	9 Rf_1-Rf_2 - 3 Rf_1- rf_2rf_2	3 $rf_1rf_1\ Rf_2$ -	1 $rf_1rf_1\ rf_2rf_2$

F= Fertile, PF= Partial fertile, S= Sterile

and is influenced by nuclear background of parental lines. Kyu and Saxena (2011) reported three types of gene interactions i.e. digenic dominant epistatic interaction, incomplete dominant epistatic and digenic recessive epistatic. Saroj *et al.* (2015) studied the segregation patterns for fertility restoration in F_2 and BC_1F_1 generations of the pigeonpea and indicated the involvement of recessive epistasis, dominant epistasis, and one cross indicated the duplicate recessive epistasis. In the present 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. The differential mode of action of restorer genes could presumably be due to the influence of the female parent genotype or to the variable expression of the weaker gene in differential segregation behaviour could also be due to the existence of certain modifiers influencing the penetrance and expressivity of the fertility restorer genes.

Conclusion

To meet the food and nutritional requirement of the ever increasing population, there is a necessity to keep upgrading the hybrid technology so that the hybrids with elevated yield and adaptations can be developed at regular interval. The information on the inheritance of fertility restoration assists in designing plan for breeding elite hybrid parents. In the present investigation, restored pollen fertility of A_4 CMS system was observed. The inter-specific derivatives involving *C. acutifolius, C. platycarpus, C. scarabaeoides, and C. lineatus* which are used to provide additional variability in hybrid breeding program also restores pollen fertility of A_4 CMS system. Thus, to breed new restorer lines, it is required to make crosses among selected diverse restorers so that desirable genotypes with preferred traits can be identified.

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