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Research Article

Inheritance study of pollen fertility restoration of CMS lines in pigeonpea

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Abstract
Segregating patterns for pollen fertility of five crosses were studied involving two CGMS lines and four restorers viz., ICPA 2043/ICP 6399, ICPA 2043/ICP 9149, ICPA 2043/ICPR 4105, ICPA 2092/ICPR 4105 and ICPA 2092/KA 91-25 in F_2 and BC_1F_1 generations. The crosses exhibited 13: 3 and 3:1 ratios, respectively in F2 and BC1F1 indicating the involvement of two dominant genes with one basic and one inhibitory gene action for fertility restoration. However, three crosses, ICPA 2092/ICP 6399, ICPA 2092/ICP 9149 and ICPA 2043/KA 91-25 recorded the ratio of 9 : 3 : 4, 12 : 3 : 1 and 9 : 7 and 1 : 1 : 2, 2 : 1 : 1 and1 : 3 in F_2 and BC_1F_1 populations, respectively and these are confirming that supplementary, masking and complimentary gene actions.

Key words:
Pigeonpea, Fertility restoration, Epistatic gene actions,

Introduction

Pigeonpea [*Cajanus cajan* (L.) Millspaugh] (2n=2x=22) is a food legume crops which belong to the family *Fabaceae* and is invariably cultivated as an annual crop. It is an often cross-pollinated (20-70%) crop, globally grown on 4.6 million hectares land in more than 20 countries with an annual production of 3.48 million tonnes and productivity being 780 kg/ha. However, in India, the area, production and productivity during 2013-2014 were 3.89 million hectares, 3.34 million tonnes and 859 kg/ha, respectively, (https://www.agricoop.nic.in).

The hybrid breeding technology which has demonstrated quantum yield jump in various field, field crops, vegetables and fruit crops with several fold increases in their productivity of the global food security in the last few decades. The availability of diverse male sterile lines and their fertility restoration have played an important role in exploiting hybrid vigor at commercial scale. Dominant fertility restoring nuclear genes are transmitted from male parent, which allow seed set on the hybrid plants. However, the expression of fertility restoration among testers in (sterility/fertility) may vary from 0 to 100 % (Saxena *et al.,* 2014). Fertility restoration of CMS-based hybrids is an integral part of breeding hybrids and the development of new hybrid parents with desirable agronomic and market preferred traits on regular intervals is essential for sustainability of hybrid technology programs.

The exploitation of hybrid vigour for enhancing the yield has paid rich dividend in most of the crop plants. The identification of genetic male sterility system (Reddy *et al*., 1978) and its utilizing resulted in release of ICPH 8, the first GMS based hybrid in pigeonpea by International Crop Research Institute for Semi- Arid Tropics (Saxena *et al.,* 1992). The importance of hybrid vigour in pigeonpea has been realized and few more hybrids such as, PPH-4, COPH-1, COPH-2, COPH 3, IPH-732, AKPH-4101 and AKPH-2022 have been released for general cultivation in some specific states. Whereas, GMS based hybrids could not be commercialized because of labour intensive seed production and seed purity as rogueing of about 50 per cent fertile plant from the female plot resulted decreased population (Reddy and Faris, 1981).

However, reported first stable cytoplasmic male sterile line, GT-288A with its maintainer, GT-288B utilized *Cajanus scarabaeoides* (A_2) as source of cytoplasm (Tikka *et al*., 1997). This cytoplasmic-genic male sterility system (CGMS) contains A line with S (rr), B line with F (rr) and R line with S/F (RR) and consequently first CGMS based hybrid SKNPH-10 (GTH-1) has been released for

cultivation in Gujarat, (Majumder, 2004). Recently, a second CMS (A_4) based hybrid, ICPH- 2671 (Pushkal) of short duration group, developed by ICRISAT for the cultivation to M. P. state (Saxena *et al*., 2013). Presently, seven CMS systems in pigeonpea have been developed by integrating the cytoplasm of wild species with the genome of cultivated species (*Cajanus cajan*) through inter-specific hybridization followed by selection and backcrossing (Saxena *et al*. 2010). Of these, A_4 CMS system derived from *C. Cajanifolius* cytoplasm (Saxena *et al*. 2005) has shown great promise 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*) in the nucleus suppress the male sterile phenotype and allow commercial exploitation of the CMS system for the production of hybrid seeds. Therefore, the present study was undertaken to assess the genetics of fertility restoration system in pigeonpea using F_1, F_2 and BC_1 generations in eight long maturing pigeonpea hybrids carrying A_4 cytoplasm.

Materials and Methods

Two cytoplasmic male sterile lines viz., ICPA 2043 and ICPA 2092 were crossed with each of about 50 diverse germplasm lines and resulting their F_1 hybrids were raised and pollen fertility of each of F_1 plants were assessed and averaged at low and high temperatures during Kharif, 2009-2010. The eight F_1 hybrids (exhibiting > 90 % pollen fertility restoration and good pod setting) involving four restorers *viz*., ICP 6399, ICP 9149, ICPR 4105 and KA 91-25 were selected and their morphological traits used as descriptors for identified for true parent as well as F_1s (Table 1). All the F_1 plants were selfed using bee proof nylon net cage (0.5 mm) to procure seeds for F_2 generation and simultaneously crossed to their respective A lines to produce BC_1 seeds besides making eight fresh crosses during second crop season 2010-2011.

The final experiments comprising of F_1s, F_2s, BC_1F_1s and parents were raised in the experimental plots at Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India, during Kharif, 2011-2012. The F_1 hybrids and parents were sown in three rows each, BC_1F_1 of 6 rows and F_2s 15 rows in each sets of segregating populations. Each plot consisted of single row of 4 m length with inter and intra row spacing of 75 × 25 cm, respectively. The rang of population of 218 - 240 plants for each F_2; 68-96 plants of BC_1F_1 and 32- 48 plants for each F_1 hybrid could be finally maintained.

For the conformation of pollen fertility/sterility, data were recorded at low (25-27 ^{0}c during November/ December) as well as high (~32 ^{0}c during March) temperature to observe the effect of temperature (thermo-sensitivity) on pollen fertility over three years (Table 2). The five fully pre-matured floral buds were picked randomly from each plant, and the anthers squashed in 2% aceto-carmine solution on a glass slide and examined under light microscope with 10x magnification. Five such microscopic fields were examined for each plant of F_1, F_2 and BC_1F_1 populations carefully. The fully stained pollen grains were identified as fertile, while the empty or partially stained pollen grains as sterile. Based on pollen fertility followed by good pod setting, the plants were classified into three categories i.e., fertile (> 90 % pollen fertility with good pod setting), partial fertile (11 – 89 % pollen fertility with poor pod setting), and sterile (0 – 10 % pollen fertility with negligible pod setting) following Kyu and Saxena (2011) with few modification. The goodness of fit to the expected ratios in F_2 and BC_1 generation was tested using chi-square test.

Results and Discussion

All the 132 F_1 hybrids evaluated on the pollen fertility restoration and pod setting rate were considered main criteria, in which 12 F_1s showed 0-10 % with negligible pod setting, 94 F_1s were revealed 11-89 % pollen fertility with poor pod setting from all 2 CMS lines respectively. However, 26 crosses were exhibited > 90 % pollen fertility with good pod setting, associated with two CMS lines *viz*., ICPA 2043 and ICPA 2092. Further, only eight selected crosses were studied in this paper.

All the lines and testers recorded 100% sterile and pollen fertility respectively and maintainers (B lines) revealed fertility ranged from 96.54 - 99.35. In the case of hybrids, all the plants were indicating fertile ranging from 94.74 to 98.67 over three years and each season had two (low and high) temperatures given in table 2, in this event incorporated dominant fertility restoring genes from the restorer parent to the hybrids. Multi-season evaluation of hybrids exhibited high stability for fertility restoration across diverse environments. The diversity study showed a large variation for important traits both at phenotypic as well as genetic levels. Saxena *et al*. 2014, had discussed the potential use of this information in hybrid pigeonpea breeding.

From the perusal of Table 3, it is obvious that percent seed setting in crossed seeds (F_0) through hand pollination, varied from 49.02 (ICPA 2043 / KA 91-25) to 58.89 (ICPA 2092 / ICP 6399) with mean of 53.23% in F_1 seeds. Whereas in BC_1F_0, varying from 31.31 (ICPA 2092 // ICPA 2092 / ICPR 4105) to 42.58 (ICPA 2092 // ICPA 2092 / ICP 6399), averaged 38.89% indicating comparatively poor seed setting in BC_1 F_0 generation. Where, 415 crossed (F_0) seeds obtained from 781 hand pollinations and 754 backcrossed (BC_1F_0) seeds from 1953 pollinations. The effects of pod setting by transferring of the masses of pollen grain mechanisms from the restorers. The dominant genes are more competitive as compare to low viability of the genes during double fertilization suggested by Liu *et al*., 2003. Earlier researchers were also been reported that influenced of nuclear background of male sterile and fertility restoring lines during inheritance study of fertility restorations of CMS lines in pigeonpea by Kyu and Saxena, (2011); Sawargaonkar *et al*. (2012).

In this ICPA 2043/ICP 6399, 203 out of 237 plants were fertile and 34 plants were male sterile. This segregation fit well to the expected ratio of 13 F : 3 S (χ^2 = 3.02; P = 0.01). In BC_1F_1 generation out of a total of 86 plants, 57 were male fertile and 29 male sterile. This fallowed a ratio of 3 : 1 (χ^2 = 3.49; P = 0.01). This segregation revealed that the restorer line ICP 6399 had two dominant loci, with one basic and one inhibitory gene action for the responsible of fertility restoration. Similarly, the F_2 and BC_1F_1 populations data along χ^2 test suggested that two dominant genes might have certain interactions with these crosses, ICPA 2043/ICP 9149, ICPA 2043/ICPR 4105, ICPA 2092/ICPR 4105 and ICPA 2092/KA 91-25 (Table 4, 5). Similar results reported in *Vicia faba* 1-2 dominant genes (Kaul, 1988) and Liu *et al.* (2003) in *Gossypium hirsutum* L.

In hybrid ICPA 2092/ICP 6399 among 232 F_2 plants grown 143 were fertile, 36 partial fertile and 53 were sterile. This segregating fit well to the expected ratio of 9 F : 3 PF : 4 S (χ^2 = 2.92; P = 0.01). In the BC_1F_1 generation, the population of 81 plants segregated in to 17 fertile, 13 partial fertile and 51 sterile and it fit well to the expected ratio of 1 F : 1 PF : 2 S (χ^2 = 5.84; P = 0.01). 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 hybrids table 4, 5. Kyu and Saxena, 2011 were also confirmed same results.

In F_2 generation of ICPA 2092/ICP 9149, 174 out of 240, 174 plants were fertile, 44 partial fertile and 22 male sterile. This segregation fit well to the expected ratio of 12 F : 3 PF :1 S (χ^2 = 3.49; P = 0.01). In back cross population, 43 out of 93 plants were fertile, 31 plants had partial fertility and 19 were sterile. This fit well to the expected ratio of 2 F : 1 PS : 1 S (χ^2 = 3.62; P = 0.01) suggesting the presence of two fertility restoration loci in the restorer parent; which interact epistatically with masking generation. 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. The other cross ICPA 2043/KA 91-25 segregated in a ratio of 9 F : 7 S (χ^2 = 3.05; P = 0.01) in F_2 generation and 1 F : 3 S (χ^2 = 2.84; P = 0.01) in back cross generation indicating the presence of two complimentary genes for restoring the fertility of mate sterile line.

The monogenic gene action, digenic dominance duplicated gene action and complementary gene action were reported that the fertility restoration in A_4 cytoplasm by Dalvi *et al*. (2008). Saxena *et al*. (2010) reported two dominant genes with one basic and one inhibitory gene action in 'ICPL 87119' hybrid. Sawargaonkar *et al*. (2012) also studied monogenic as well as digenic control of fertility restoration and suggested that fertility influenced by nuclear background of parental lines. Hossain *et al.* (2010) suggested the differential segregation behaviour could also be due to the existence of certain modifiers influencing the penetrance and expressivity of the fertility restorer genes.

Further studies are necessary to confirm the genetic systems underlying the fertility restoration of male sterility system with inbred lines.

To conclude, the inheritance of fertility restoration in CMS lines in specific crosses of pigeonpea influenced by the nuclear background of male sterile and fertility restoring lines. The differential behavior of three fertility restorer lines ICP 6399, ICP 9149 and KA 91-25 in six different crosses viz., ICPA 2043/ICP 6399, ICPA 2092/ICP 6399, ICPA 2043/ICP 9149, ICPA 2092/ICP 9149 and ICPA 2043/KA 91-25, ICPA 2092/KA 91-25, were attributed to the interactions of different nuclear genes of the two female parents.

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Table 1. The origin/source of different cytoplasmic male sterile and restorers/ varieties used in study

S. No.	CMS/restorer	Days to maturity	A_4 Cytoplasm	Origin/source	Descriptors
	CMS Lines				
1.	ICPA 2043	-	*Cajanus cajanifolius*	ICRISAT, Hyderabad	Compact plant type, Medium tall plant height, Yellow flower, Pod Green, Brown seed colour
2.	ICPA 2092	-	”	”	Spreading plant type, Medium tall plant height, Yellow flower, Pod GPS, Redish brown Seed colour
	Restorer Lines				
3.	ICP 6399	237.41	*Cajanus cajan*	ICRISAT, Hyderabad	Compact plant type, Medium tall plant height, Yellow flower, Pod GPS, Whitish yellow seed colour
4.	ICP 9149	237.54	”	”	Compact plant type, Medium tall plant height, Yellow flower, pod Dark GPS, Brown seed colour
5.	ICPR 4105	237.43	”	”	Compact plant type, Tall plant height, Yellow flower, pod GPS, Redish brown seed colour
6.	K A 91-25	238.33	”	CSAU, Kanpur	Compact plant type, tall plant height, Yellow flower, pod GPS, Redish brown seed colour

Table 2. Mean of pollen fertility of F_1 hybrids and parents at low and high temperatures over three years

S. No.	*Hybrids*	Pollen fertility (%)					
		(2009-2010)		(2010-2011)		(2011-2012)	
		November/ December	March	November/ December	March	November/ December	March
		Low temp. (27.08 ^{0}C)	High temp. (32.15 ^{0}C)	Low temp. (26.18 ^{0}C)	High temp. (32.83 ^{0}C)	Low temp. (24.97 ^{0}C)	High temp. (31.90 ^{0}C)
1.	ICPA 2043/ICP 6399	96.40	97.06	95.44	95.66	96.57	96.60
2.	ICPA 2092/ICP 6399	96.53	97.86	97.05	97.40	96.21	96.87
3.	ICPA 2043/ICP 9149	95.66	95.26	96.44	97.77	97.06	97.50
4.	ICPA 2092/ICP 9149	97.42	96.66	96.20	96.79	95.81	97.07
5.	ICPA 2043/ICPR 4105	97.38	95.36	97.81	97.56	97.08	98.08
6.	ICPA 2092/ICPR 4105	95.67	98.65	98.01	98.35	98.04	98.67
7.	ICPA 2043/KA 91-25	94.74	97.26	97.38	98.04	96.93	98.27
8.	ICPA 2092/KA 91-25	96.09	95.36	98.20	98.54	96.87	98.24
	Lines						
5.	ICPA 2043	0.00	0.00	0.00	0.00	0.00	0.00
6.	ICPA 2092	0.00	0.00	0.00	0.00	0.00	0.00
7.	ICPB 2043	98.70	98.57	98.30	98.13	99.35	97.36
8.	ICPB 2092	97.99	98.79	97.97	97.47	98.51	96.54
	Testers						
9.	ICP 6399	100	100	100	100	100	100
10.	ICP 9149	100	100	100	100	100	100
11.	ICPR 4105	100	100	100	100	100	100
12.	KA 91-25	100	100	100	100	100	100

Table 3. Description of % success crossed seeds harvested through manual pollinations

S. No.	Crosses	Bud Pollinated	Seeds harvested	% Success
	F_1 hybrids			
1.	ICPA 2043 / ICP 6399	98	52	53.06
2.	ICPA 2092 / ICP 6399	90	53	58.89
3.	ICPA 2043 / ICP 9149	91	49	53.85
4.	ICPA 2092 / ICP 9149	97	51	52.58
5.	ICPA 2043 / ICPR 4105	106	56	52.83
6.	ICPA 2092 / ICPR 4105	98	53	54.08
7.	ICPA 2043 / KA 91-25	102	50	49.02
8.	ICPA 2092 / KA 91-25	99	51	51.52
	Total/ mean	781	415	53.23
	Test crosses			
9.	ICPA 2043 // ICPA 2043 / ICP 6399	225	89	39.56
10.	ICPA 2092 // ICPA 2092 / ICP 6399	209	89	42.58
11.	ICPA 2043 // ICPA 2043 / ICP 9149	241	98	40.66
12.	ICPA 2092 // ICPA 2092 / ICP 9149	248	99	39.92
13.	ICPA 2043 // ICPA 2043 / ICPR 4105	249	100	40.16
14.	ICPA 2092 // ICPA 2092 / ICPR 4105	297	93	31.31
15.	ICPA 2043 // ICPA 2043 / KA 91-25	238	97	40.76
16.	ICPA 2092 // ICPA 2092 / KA 91-25	246	89	36.18
	Total/ mean	*1953*	*754*	*38.89*

Table 4. Pollen fertility of F_2 segregating populations

S. No.	Crosses	No of Plants				Segregation ratio
		Total	Fertile	Partial fertile	Sterile	
1.	ICPA 2043/ICP 6399	237	203	0	34	$\chi^2_{13:3} = 3.02^{NS}$
2.	ICPA 2092/ICP 6399	232	143	36	53	$\chi^2_{9:3:4} = 2.92^{NS}$
3.	ICPA 2043/ICP 9149	218	166	0	52	$\chi^2_{13:3} = 3.73^{NS}$
4.	ICPA 2092/ICP 9149	240	174	44	22	$\chi^2_{12:3:1} = 3.49^{NS}$
5.	ICPA 2043/ICPR 4105	236	201	0	35	$\chi^2_{13:3} = 2.38^{NS}$
6.	ICPA 2092/ICPR 4105	221	189	0	32	$\chi^2_{13:3} = 2.65^{NS}$
7.	ICPA 2043/KA 91-25	219	136	0	83	$\chi^2_{9:7} = 3.05^{NS}$
8.	ICPA 2092/KA 91-25	226	173	0	53	$\chi^2_{13:3} = 3.28^{NS}$

Non- significant at P= 0.05 & 0.01 level respectively.

Table 5. Pollen fertility of testcross populations

S. No.	Test crosses	No of Plants				Segregation ratio
		Total	Fertile	Partial fertile	Sterile	
1.	ICPA 2043//ICPA 2043/ICP 6399	86	57	0	29	$\chi^2_{3:1} = 3.49^{NS}$
2.	ICPA 2092//ICPA 2092/ICP 6399	81	17	13	51	$\chi^2_{1:1:2} = 5.84^{NS}$
3.	ICPA 2043//ICPA 2043/ICP 9149	89	61	0	28	$\chi^2_{3:1} = 1.98^{NS}$
4.	ICPA 2092//ICPA 2092/ICP 9149	93	43	31	19	$\chi^2_{2:1:1} = 3.62^{NS}$
5.	ICPA 2043//ICPA 2043/ICPR 4105	92	61	0	31	$\chi^2_{3:1} = 3.71^{NS}$
6.	ICPA 2092//ICPA 2092/ICPR 4105	82	54	0	28	$\chi^2_{3:1} = 3.66^{NS}$
7.	ICPA 2043//ICPA 2043/KA 91-25	92	30	0	62	$\chi^2_{1:3} = 2.84^{NS}$
8.	ICPA 2092//ICPA 2092/KA 91-25	81	68	0	13	$\chi^2_{3:1} = 3.46^{NS}$

Non- significant at P= 0.05 & 0.01 level respectively.