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Identification of fertility restorers, stable combiners and heterotic combinations across environments in pigeonpea [*Cajanus cajanifolius* (L.) Millsp.] in India

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Abstract: Efficient and stable cytoplasmic genic male sterility system is a prerequisite for commercial exploitation of heterosis in any crop improvement program. The present investigation was undertaken to identify fertility restorers, stable combiners and best heterotic crosses in pigeonpea [*Cajanus cajanifolius* (L.) Millsp]. Totally, 75 crosses were developed by using three A_4 cytoplasm-based male sterile lines *viz*., ICPA 2047, ICPA 2092 and ICPA 2089, and 25 diverse inbred lines. The pollen fertility assay identified 15 good restorers with 85-95% fertility status. The 45, best restored combinations identified were assessed across four seasons (August and September, 2012 and 2013) for their stable heterosis and general and specific combining ability to identify parents possessing desirable genes. Variance for combining ability was significant ($p<.05$) for lines, testers and line × tester effects. Among the three male sterile lines, ICPA 2047 was stable with a significant general combining ability for most of the traits followed by ICPA 2092, while the general combining ability effects among males was high across seasons for ICPL 20205 and ICPL 20177 ($p<0.05$). The cross combination of ICPA 2089 × ICPL 20205 had desirable genes for days to the first flowering, 50% flowering and maturity. For yield related traits, cross combinations of ICPA 2047 × ICPL 20128, ICPA 2092 × ICPL 11376 and ICPA 2047 × ICPL 20107 had significant specific combining ability indicating their usability in development of hybrids ($p<0.05$). With respect to the heterotic ability of crosses, crosses involving parents ICPA 2089 as female and ICPL 88034 and ICPL 81-3 as males showed negative heterosis for flowering and maturity. For yield related traits, crosses with ICPA 2047 as the female parent and ICPL 20128, ICPL 20177 and ICPL 20096 as the male parents showed significant positive heterosis ($p<0.05$). The identified parents with good combining ability and cross combination`s with significant heterosis could be utilized in producing high yielding hybrids in pigeonpea.

***Keywords*:** CGMS, combining ability, fertility restoration, heterosis, pigeonpea

Introduction

Pigeonpea [*Cajanus cajanifolius* (L.) Millsp] is an important grain legume crop of rainfed agriculture in the semi-arid tropics. It is grown widely in the Indian subcontinent. The gross cultivated area under pigeonpea in India is 3.86 million ha with a total production of 2.65 million mt and a productivity of 686.5 kg ha^{-1}. In spite of the efforts made by the pigeonpea crop researchers for more than

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five decades, the progress in the genetic improvement of yield in this crop is limited.

Hybrid pigeonpea is one of the potential methods to break this yield stagnation. The stable male-sterility system in conjunction with natural out-crossing will make the hybrid pigeonpea production easy and affordable. Effective utilization of a male-sterility system in hybrid breeding can be accomplished firstly by identification of stable restorers for the concerned male sterile lines over different environments. Secondly, it is also essential to narrow down male sterile lines, maintainer and restorer lines having high general combining ability for yield and its attributing traits and hybrid with desirable specific combining ability. Thirdly, is to identify a stable hybrids and ideal environment to realize the potential yield of pigeonpea. The present study was thus carried out to identify fertility restorers, stable combiners and best heterotic crosses in pigeonpea.

Materials and Methods

The experimental material for the restoration study comprised of six A_4 cytoplasm-based CMS lines of pigeonpea *viz*., ICPA 2047, ICPA 2092, ICPA 2089 (developed at the International Crops Research Institute for Semi-Arid Tropics, Hyderabad, India), BRG 1A, BRG 3A, Hy3cA (developed at the University of Agricultural Sciences, Bangalore, India) and 61 pollen parents. A total of 75 cross combinations were produced in line × tester mating design with three female (ICPA 2047, ICPA 2092 and ICPA 2089) and 25 male parents. Similarly, 108 combinations were produced between three female (BRG 1A, BRG 3A and Hy3cA) and 36 male parents. All cross combinations were evaluated along with parents and two checks (ICPH 2671 and ICP 8863) in a randomized complete block design (RCBD) with two replicates in 2012. For testing the pollen fertility in the hybrids, a 2% acetocarmine stain was used and pollens were examined under light microscope to differentiate the fertile and sterile pollens. The mean per cent pollen fertility of hybrids was calculated (Equation 1).

$$\text{Pollen fertility (\%)} = \frac{\text{No. of fertile (round and stained) pollens}}{\text{Total number of pollen grains studied}} \times 100 \quad \text{------ (Equation 1)}$$

Based on the fertility restoration in the hybrids, 45 hybrid combinations involving three male sterile lines (ICPA 2047, ICPA 2092 and ICPA 2089) and 15 restorers were selected for studies leading to combining ability and heterosis. The 45 hybrids along with the check (ICPH 2671) were planted in a RCBD with two replicates during four seasons in August and September of 2012 and 2013. Observations were recorded on nine quantitative traits on randomly selected five competitive plants on each of the hybrids for estimating combining ability and heterosis. The overall status of hybrids across of the traits was determined using the method proposed by Arunachalum *et al.* (1984).

Results and Discussion

The frequency of restorers for different A_4 cytoplasm-based male sterile lines is shown in Tables 1 and 2. In total, 56 pollen parents were found to be restoring fertility of hybrids (more than 80%). Among the different cross combinations studied, 14 genotypes (56%) were found to be the restorer for ICPA 2047, 12 genotypes (48%) for ICPA 2092, eight genotypes (32%) for ICPA 2089, six genotypes (17 %) for BRG 3A and eight genotypes each with 22% frequency restored fertility in the hybrid combinations with BRG 1Aand Hy3cA. Basha *et al.* (2008), Saxena (2009) and Makelo *et al.* (2013) reported such variations when tested under varying environmental conditions.

Table 2 indicates that 12 genotypes were the common restorers for ICPA 2047 and ICPA 2092, eight each for ICPA 2047 and ICPA 2089, ICPA 2092 and ICPA 2089, ICPA 2047, ICPA 2092 and ICPA 2089, three for BRG 1A and BRG 3A; four each for BRG 1A and Hy3Ca; BRG 3A and Hy3cA, and two for BRG 1A, BRG 3A and Hy3cA. The genotype ICPL 20093 was the common restorer for all the six male sterile lines.

Table 1. Frequency of restorers of six A_4 cytoplasm based male sterile lines in pigeonpea

CMS Lines	Number of pollen parents	No. of restorers with >80% pollen fertility	Frequency of restorers (%)
ICPA 2047	25	14	56
ICPA 2092	25	12	48
ICPA 2089	25	8	32
BRG 1 A	36	8	22
BRG 3A	36	6	17
Hy 3C A	36	8	22

The 45 hybrid combinations were analyzed for combining ability of their parents and heterosis across four seasons. The analysis of variance (Table 3) revealed significant differences between gca effects of CMS lines for all the traits except for pods per plant. In case of pollen parents, effects of gca differed significantly for days to maturity, plant height, secondary branches and 100 seed weight. The male sterile line ICPA 2089 was the best general combiner with negative and significant gca effects for days to 50% flowering and maturity, while ICPA 2047 was a desirable combiner for yield and its related traits (Table 4).

Table 2. Common restorers for six A_4 cytoplasm based male sterile lines in pigeonpea

CMS Lines	No. of restorers	Restorers
ICPA 2047 & ICPA 2092	12	ICPL 161, ICPL 11376, ICPL 20093, ICPL 20096, ICPL 20098, ICPL 20108, ICPL 20128, CPL 20166, ICPL 20205, ICPL 81-3 , ICPL 87119 & ICPL 88039
ICPA 2047 & ICPA 2089	8	ICPL 11376, ICPL 20093, ICPL 20096, ICPL 20098, ICPL 20166, ICPL 20205, ICPL 81-3 & ICPL 87119
ICPA 2092 & ICPA 2089	8	ICPL 11376, ICPL 20093, ICPL 20096, ICPL 20098, ICPL 20166, ICPL 20205, ICPL 81-3 & ICPL 87119
ICPA 2047, ICPA 2092 & ICPA 2089	8	ICPL 11376, ICPL 20093, ICPL 20096, ICPL 20098, ICPL 20166, ICPL 20205, ICPL 87119 & ICPL 81-3
BRG 1A & BRG 3A	3	TS 3R, ICPL 20093 & BRGL 13-171
BRG 1A & Hy 3C A	4	GCB 126, ICPL 81-3, ICPL 20093 & BRGL 13-171
BRG 3A & Hy 3C A	4	ICPL 20093, ICPL 20116, BRGL 13-167 & BRGL 13-171
BRG 1A , BRG 3 A & Hy 3C A	2	ICPL 20093 & BRGL 13-171

Amongst the pollen parents, ICPL 20128 and ICPL 161 have negative and significant gca effects on days to 50% flowering and maturity, while ICPL 20128 and ICPL 20177 showed significant and positive gca effects on yield and its related traits ($p<0.05$). Dalvi *et al*. (2008), Yadav *et al.* (2008) and Sameerkumar *et al*. (2009) observed similar results with parents having high gca effects, which has ultimately resulted in hybrids with superior sca effects.

The estimates of specific combining ability effects of 45 hybrids are summarized in Table 5. The hybrid ICPA 2047 × ICPL 20098 and ICPA 2092 × ICPL 88034 were identified as the best specific combiners in desirable direction for days to 50% flowering and maturity. The hybrid combinations, ICPA 2092 × ICPL 20128 and ICPA 2089 × ICPL 20205 for plant height; ICPA 2047 × ICPL 20098 and ICPA 2092 × ICPL 20108 for primary branches, ICPA 2089 × ICPL 161 and ICPA 2089 × ICPL 81-3 for secondary branches, ICPA 2047 × ICPL 20128 and ICPA 2047 × ICPL 161 for number of pods per plant, ICPA 2047 × ICPL 20128 and ICPA 2047 × ICPL 20093 for number of seeds per pods, ICPA 2089 × ICPL 88034 and ICPA 2089 × ICPL 88039 for 100 seed weight were the best specific combinations in desirable combinations.

For seed yield per plant, the hybrids ICPA 2092 × ICPL 11376 and ICPL ICPA 2047 × ICPL 20128 were the best specific combinations. Dalvi (2008), Yadav *et al*. (2008) and Sameerkumar *et al*. (2009) also reported similar results that could lead to hybrids with superior sca effects.

Table 3. Analysis of variance for combining ability effects across environments in pigeonpea

Sources of variation	df	Days to 50% flowering	Days to maturity	Plant height (cm)	Primary branches/ plant	Secondary branches/ Plant	Pods/plant	Seeds/ pod	100 seed weight (g)	Seed yield/plant (g)
Replication	1	2939.0**	4.0	4.0	24.3**	17.9*	357.8	0.1	2.0	103.7
Hybrids	44	4296.5**	1726.5**	1726.5**	10.5**	21.5**	18939.9**	0.2**	4.3**	365.1**
Line	2	65632.6**	26372.0**	26372.0**	93.8**	247.9**	46708.3	0.7**	16.1**	2167.1**
Tester	14	1607.7	1392.4**	1392.4**	9.3	20.3**	24129.1	0.2	6.7**	312.8
Line * Tester	28	1259.7**	133.1**	133.1**	5.2**	5.9**	14361.9**	0.1**	2.3**	262.6**
Env * Hybrids	132	2099.9**	266.5**	266.5**	5.6**	9.9**	4179.6**	0.04	2.1**	217.8**
Env * Line	6	21415.2**	759.4**	759.4**	34.8**	31.3**	10909.5*	0.07	1.7	355.4
Env * Tester	42	1190.8	242.9	242.9	4.1	10.1	2337.8	0.04	2.3	155.9
Error	176	158.5	10.2	10.2	2.3	2.9	625.7	0.05	0.9	42.9
Var (GCA)		464.8	192.7	112.8	0.7	1.8	484.5	0.01	0.2	16.7
Var (SCA)		138.6	15.4	13.7	0.3	0.4	1728.2	0.01	0.2	28.1
Var (GCA)/Var (SCA)		3.35	12.49	8.21	2.19	4.55	0.28	0.77	0.8	0.59

*Significant at p=0.05, ** Significant at p=0.01

Table 4. Estimates of gca effects of parents across four environments for nine traits in pigeonpea

Parental lines	Characters	Genotypes	gca effects
CMS Lines (Seed parents)	Days to 50 % flowering	ICPA 2089	-13.03
	Days to maturity	ICPA 2089	-16.99
	Plant height (cm)	ICPA 2047	18.48
	Primary branches/plant	ICPA 2047	0.63
	Secondary branches/plant	ICPA 2047	0.74
	Pods/plant	ICPA 2047	21.03
	Seeds/Pod	ICPA 2047	0.07
	100 Seed Weight (g)	ICPA 2047	0.29
	Seed yield/ plant (g)	ICPA 2047	4.46
Pollen Parents	Days to 50% flowering	ICPL 20128	-5.62
	Days to maturity	ICPL 161	-11.46
	Plant height (cm)	ICPL 20128	12.96
	Primary branches/ plant	ICPL 20177	0.71
	Secondary branches/plant	ICPL 20108	1.34
	Pods/plant	ICPL 20177	68.82
	Seeds/Pod	ICPL 20098	0.12
	100 Seed Weight (g)	ICPL 20093	0.80
	Seed yield/ plant (g)	ICPL 20177	5.02

Table 5. Best three hybrids based on sca effects and their *per se* performance for nine traits in pigeonpea

Characters	Hybrids	sca	Mean	Standard heterosis over ICPH 2671
Days to 50 % flowering	ICPA 2047 × ICPL 20098	- 6.09	100.50	15.85
	ICPA 2047 × ICPL 20128	- 5.42	90.23	4.30
	ICPL 2092 × ICPL 88034	- 5.02	89.75	3.46
Days to maturity	ICPA 2092 × ICPL 88034	- 6.13	130.25	- 3.25
	ICPA 2047 × ICPL 20098	- 5.93	152.00	12.91
	ICPA 2089 × ICPL 20205	- 4.80	128.25	- 4.74
Plant height (cm)	ICPA 2092 × ICPL 20108	19.38	177.85	17.30
	ICPA 2089 × ICPL 20205	19.13	144.92	- 4.42
	ICPA 2047 × ICPA 88034	17.11	178.20	17.53
Primary branches/ Plant	ICPA 2092 × ICPL 20108	0.88	9.58	33.27
	ICPA 2047 × ICPL 20098	0.95	10.27	42.82
	ICPA 2047 × ICPL 20205	1.26	9.78	36.03
Secondary branches/ Plant	ICPA 2092 × ICPL 20128	1.18	5.67	116.73
	ICPA 2089 × ICPL 81-3	1.51	1.03	- 60.80
	ICPA 2089 × ICPL 161	1.83	1.69	- 35.28
Pods/Plant	ICPA 2089 × ICPL 20107	59.07	225.09	19.09
	ICPA 2047 × ICPL 161	64.29	244.85	29.55
	ICPA 2047 × ICPL 20128	66.05	285.40	51.00
Seeds/Pod	ICPA 2047 × ICPL 20128	0.14	3.80	6.37
	ICPA 2089 × ICPL 20093	0.14	3.80	6.37
	ICPA 2047 × ICPL 20107	0.20	3.93	9.94

100 Seed Weight (g)	ICPA 2089 × ICPL 88039	0.73	10.66	- 4.76
	ICPA 2092 × ICPL 20098	0.76	10.95	3.23
	ICPA 2089 × ICPL 88034	0.79	10.62	- 5.17
Seed yield/plant (g)	ICPA 2089 × ICPL 20166	6.19	37.34	11.81
	ICPA 2047 × ICPL 20128	11.67	54.72	63.85
	ICPA 2092 × ICPL 11376	14.73	50.73	51.92

Amongst the 45 hybrids, ICPA × ICPL 20128 and ICPA 2092 × ICPL 11376 were the most heterotic over the standard check ICPH 2761 for seed yield per plant. Sekhar *et al.* (2004) and Wanjari *et al.* (2007) reported substantial heterosis for grain yield and other economic characters over the best checks in pigeonpea.

Twenty out of 45 hybrids recorded an overall high sca status over environments (Table 6). From these 20 hybrids, which showed higher overall sca status, four were of H × H, five with H × L, four with L × H and remaining seven with L × L combinations. In addition to determining the overall gca status of parents and sca status of hybrids, it is important to determine overall heterotic status of hybrids across the traits (Table 7). It is evident from the table 7 that 23 out of 45 hybrids had high (H) heterotic status and the remaining hybrids had low (L) overall heterotic status across the traits.

Table 6. Overall specific combining ability status of hybrids for nine traits in pigeonpea

Lines/ Testers with overall gca	ICPA 2047 (H)		ICPA 2092 (L)		ICPA 2089 (L)	
	Total score	Over all heterotic status	Total score	Over all heterotic status	Total score	Over all heterotic status
ICPL 20108 (L)	151	L	371	H	245	L
ICPL 20166 (L)	201	L	239	L	356	H
ICPL 11376 (L)	207	L	376	H	139	L
ICPL 161 (L)	328	H	174	L	222	L
ICPL 87119 (H)	281	H	285	H	198	L
ICPL 20205 (H)	180	L	249	L	305	H
ICPL 20098 (L)	340	H	184	L	258	H
ICPL 20096 (L)	258	H	211	L	272	H
ICPL 20093 (H)	243	L	226	L	316	H
ICPL 81-3 (L)	211	L	299	H	239	L
ICPL 20128 (H)	399	H	198	L	164	L
ICPL 88039 (L)	214	L	349	H	198	L
ICPL 20107 (H)	209	L	188	L	363	H
ICPL 88034 (H)	265	H	268	H	231	L
ICPL 20177 (H)	256	H	183	L	336	H

Final norm: 253, (H) - High overall general combiner, (L) - Low overall general combiner, H - High overall specific combination, L - Low overall specific combination

Table 7. Overall heterotic status of hybrids for nine traits in pigeonpea

Lines/ Testers with overall gca	ICPA 2047 (H)		ICPA 2092 (L)		ICPA 2089 (L)	
	Total score	Over all heterotic status	Total score	Over all heterotic status	Total score	Over all heterotic status
ICPL 20108 (L)	164	L	336	H	323	H
ICPL 20166 (L)	155	L	270	H	335	H
ICPL 11376 (L)	147	L	313	H	166	L
ICPL 161 (L)	272	H	260	H	300	H
ICPL 87119 (H)	231	L	323	H	313	H
ICPL 20205 (H)	142	L	241	L	292	H
ICPL 20098 (L)	182	L	163	L	229	L
ICPL 20096 (L)	187	L	250	L	301	H
ICPL 20093 (H)	119	L	235	L	227	L
ICPL 81-3 (L)	115	L	202	L	220	L
ICPL 20128 (H)	385	H	277	H	356	H
ICPL 88039 (L)	178	L	304	H	260	H
ICPL 20107 (H)	199	L	237	L	344	H
ICPL 88034 (H)	311	H	331	H	363	H
ICPL 20177 (H)	241	L	268	H	318	H

Final norm: 253; (H) - Overall high general combiner, (L) - Overall low general Combiner. H - Overall high specific combination, L - Overall low specific combination

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