

Original Research Article

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Study of Heterosis and Pollen Fertility in CGMS Based Pigeonpea [*Cajanus cajan* (L.) Millspaugh] Hybrids

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ABSTRACT

Thirty CGMS-based pigeonpea [*Cajanus cajan* (L.) Millspaugh] hybrids were synthesized by crossing six CMS lines with five 'R' lines and evaluated to study yield potential with the performance of their R-lines. Result indicated that the male sterile lines exhibited 100 per cent pollen sterility and R- line acts as good restorer and pollen fertility varied from 98 to 100 per cent. All crosses exhibited pollen fertility ranged from 92 to 100 per cent. Most of the hybrids showed standard heterosis in desired direction for yield and its contributing traits with high fertility restoration. The range of standard heterosis over GTH 1 for seed yield per plant was from -27.29 (CMS GT 301 A x GTR 52) to 69.79 per cent (CMS GT 603 A x GTR 52). The best cross combinations for seed yield were CMS GT 603 A X GTR 52 (69.79 %), CMS GT 603 A X GTR 23 (64.59 %), CMS GT 288 A X GTR 95 (57.22 %) and CMS GT 603 A X GTR 95 (53.76 %), CMS GT 601 A X GTR 95 (49.41 %), CMS GT 33 A X GTR 18 (47.69 %), CMS GT 603 A X GTR 8 (46.81 %), CMS GT 601 A X GTR 52 (37.30 %), CMS GT 302 A X GTR 8 (33.39 %) and CMS GT 301 A X GTR 95 (28.61 %). These hybrids had high per se performance for grain yield, more standard heterosis with one or more its contributing traits viz. number of pods per plant, biological yield, leaf area and harvest index and having high pollen fertility status suggested that these hybrids can be directly exploited commercially after evaluating their performance in wide range of environment. These crosses can also be used to throw-off transgressive segregants for the improvement of yield and its attributing traits.

Keywords

CMS,
Pollen fertility
restorers,
Heterosis,
Pigeonpea.

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Introduction

Pigeonpea [*Cajanus cajan* (L.) Millsp.] Is an environment friendly crop, which is a staple food across the country and plays an important role in national economic and nutritional security with qualities of improving soil fertility and structure? It is an important food legume of semi-arid tropical regions of Asia, Africa and the Caribbean islands, where it is grown on over 5 m ha (FAO, 2013). Productivity of pigeonpea worldwide in comparisons to cereal is very

low and stagnant due to several biotic and abiotic stress and in spite of decades of research and development programs, the mean productivity of the crop could not cross the barrier of 800 kg/ha. Hybrid breeding technology, developed by ICRISAT to break the yield plateau which is based on cytoplasmic nuclear male sterility (CMS) and natural out-crossing. The first stable CMS line for commercial exploitation could be developed by Tikka *et al.*, (1997), GT-288A

with its maintainer, GT-288B utilizing as A2 cytoplasm source. Later pollen fertility restorer lines were identified (Chauhan *et al.*, 2004, Acharya *et al.*, 2005). For getting good yield, male fertility restoration of hybrid plant is important. According to Kaul (1988) once a fertility restorer (R-) line is crossed with male sterile (A-) plant, the dominant fertility restoring nuclear gene produces certain proteins in F1 plants and thus repairs the defective mitochondrial genome of the plant to produce male fertile hybrid plants. In pigeonpea two dominant genes (Rf1 and Rf2) have been identified (Saxena *et al.*, 2011), which impart fertility restoration to the hybrid plants. This cytoplasmic-genic male sterility system 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 (Patel *et al.*, 2004 and Majmudar *et al.*, 2004). In the primary gene pool the frequency of fertility restoring genes is fairly high and so far over 150 good restorers have been identified in different maturity groups (Saxena *et al.*, 2014), heterosis using cms lines and pollen fertility restorer lines for grain yield studied by Patel and Tikka (2014a,b).

The hybrid technology is based on three major components namely, male sterility and its genetic maintenance system, stable fertility restoration, and cost effective mass pollination mechanism. The development of stable CMS systems in pigeonpea is a boon to the breeders and it has provided a platform to enhance the pace of research and development of hybrid pigeonpea. The utilization of CMS system for the development of commercial hybrids in pigeonpea would be possible of improvement of seed yield by developing hybrids or by selecting transgressive segregants from the crosses showing high heterotic response as pollen fertile restorer parent.

Materials and Methods

The thirty hybrids obtained through hand pollination during kharif 2014 at Main Pulses Research Station, Sardarkrushinagar Dantiwada Agricultural University, SardarKrushinagar using newly developed six cytoplasmic male sterile lines and five diverse restorers as pollinators in a line x tester mating design (Table 1). The experiments conducted during kharif 2015. The latitude and longitude were 24° 12' N and 72° 12' E. The altitude and soil type were 154.5 m and loamy sand, for these location. The experimental materials comprised of six cytoplasmic male sterile line used as fertile counterpart, five pollen fertility restorer line as male parents, thirty synthesized hybrids and standard check GTH 1 and evaluated using randomized block design with three replications for each location. Each genotype was represented by a single row plot of 4.0 m length. The inter and intra row distances were 60 and 20 cm, respectively which accommodated 20 plants per plot. All the agronomical practices and plant protection measures were followed for raising the good crop. Observations were recorded on five randomly selected competitive plants of each genotype in each replication for various characters i.e. plant height (PH) (cm), number of branches per plant (BP), number of pods per plant (PP), pod length (PL) (cm), Number of seeds per pod (SP), 100-seed weight (g) (TW), seed yield per plant (g) (SY), biological yield per plant (g) (BY). Days to flower (DF) on the basis of 50 % plants of each genotype flowered, days to maturity (DM) on the basis of 80 % plants of each genotype matured were recorded. The protein content (PC) was estimated in percentage by using Nuclear Magnetic Resonance Technique (Tiwari *et al.*, 1974). Harvest Index was calculated by using following formula [(Economic yield/Biological yield) x 100]. The replication wise mean values were

used in statistical analysis. The replication wise mean value of each genotype for various characters was used for statistical genetical analysis. Heterosis was estimated as per cent increase or decrease in the mean value of F1 hybrid over standard check *i.e.*, standard heterosis Meredith and Bridge (1972) for each character. The pollen fertility/sterility observations were recorded on parental lines ('A' lines and male parents) during crossing season and for F1 and check GTH 1 were recorded during evaluating season at the initiation of flowering stage. The fertility status was determined. The test for fertility and sterility of pollen grains was done as per aceto-carmin stain method by Alexander, 1969.

The test comprised staining pollen grains in 2 gm carmine solution was prepared by dissolving in hot 45% glacial acetic acid, boil for half an hour and cool and filter. Five well developed flower buds were collected randomly from different parts of each plant at the time of anthesis (9-10 AM). From each bud, the anthers were collected on a glass slide and crushed with a drop of 2% aceto-carmin stain and examined under a light microscope. The mean value of pollen fertility/sterility of five plants was considered as pollen fertility/sterility (%) for that genotype (Saxena *et al.*, 2011).

Results and Discussion

The analysis of variance for all the characters under study was presented in Table 1. There were significant differences among the parents for all the characters except protein content in parents. This indicated the presence of adequate amount of variability in parents (lines and testers) for most of the characters under studied. Mean sum of squares due to lines were significant for all the characters except seed yield, branches per plant and biological yield. Mean sum of squares due to

testers were significant for all the characters except number of pods and pod length. Further, mean sum of squares due to hybrids and parent vs. hybrids were significant for all the characters except for number of seeds per pod, branches per plant and protein content, which indicated the presence of enormous heterosis for these traits. Mean sum of squares due to line vs. tester significant for all characters except 100 seed weight.

Heterosis estimated over check (Standard Heterosis)

The main aim of plant breeder is to evolve high yielding varieties. The yield is the attribute that receive greatest importance. It is a complex trait associated with number of component traits which are under polygenic control. The perusal of the results revealed (Table 2) that thirteen hybrids exhibited significant and positive standard heterosis. The standard heterosis ranged from -27.29 (CMS GT 301 A x GTR 52) to 69.79 per cent (CMS GT 603 A x GTR 52).

Three most positive heterotic crosses for GTH 1 in descending order were CMS GT 603 A X GTR 52 (69.79 %) and CMS GT 603 A X GTR 23 (64.59 %) and CMS GT 288 X GTR 95 (57.22). Similar findings were also evident by the findings of Patel and Tikka (2008), Acharya *et al.*, (2009), Dheva *et al.*, (2009), Gite *et al.*, (2009), Phad *et al.*, (2009), Sameer Kumar *et al.*, (2009), Sarode *et al.*, (2009), Chadirakala *et al.*, (2010), Shoba and Balan (2010), Gupta *et al.*, (2011) and Lay *et al.*, (2011).

Early flowering and maturity is one of the desirable traits in hybrid pigeonpea as it helps in escaping drought. Majority of hybrids showed late maturity. The estimates of standard heterosis for days to flowering revealed (Table 2) that five hybrids for significant heterosis in desired direction

(negative) with a range from -13.03 (CMS GT 33 A X GTR 95) to 22.18 per cent CMS GT 301 A X GTR 18) and for days to maturity ranged from -3.59 (CMS GT 33 A X GTR 23) to 18.90 per cent (CMS GT 601 A X GTR 23). Heterosis in both negative and positive direction for days to flowering had also evident by Kumar and Srivastva (1998), Wankhade *et al.*, (2005), Baskaran and Muthiah (2006), Wanjari *et al.*, (2007), Patel and Tikka (2008), Sarode *et al.*, (2009), Chandirakala *et al.*, (2010) and Vaghela *et al.*, (2011).

The estimates of heterosis for plant height revealed (Table 2) that majority of hybrids exhibit taller stature over standard check. The standard heterosis ranged from -4.47 per cent (CMS GT 33 A X GTR 52) to 41.76 per cent (CMS GT 603 A X GTR 52). Similar findings were also recorded by Rana (1990), Aghav *et al.*, (1997), Chandirakala and Raveendran (2002) and Chandirakala (2010) *et al.*, for plant height.

The standard heterosis for number of branches per plant varied from -21.41 (CMS GT 33 A X GTR 18 and CMS GT 601 A X GTR 52) to 27.31 per cent (CMS GT 301 A X GTR 95). The hybrids with positive heterosis for number of pods per plant are desirable to increase yield. The standard heterosis ranged from -36.00 (CMS GT 301 A X GTR 52) to 15.97 per cent (CMS GT 301 A X GTR 95). Results were found agreement with Phad *et*

al., (2009) and Shoba and Balan (2010) for number of branches per plant and number of pods per plant.

The results presented in table 2 revealed that out of 30 hybrids, none of the hybrids evinced significant and positive standard heterosis for number of seeds per pod. The range of variation of standard heterosis from -24.21 (CMS GT 301 A x GTR 18) to 1.69 per cent (CMS GT 301 A X GTR 52, CMS GT 302 A X GTR 18, CMS GT 33 A X GTR 95, CMS GT 288 A X GTR 23, CMS GT 603 A X GTR 18, CMS GT 603 A X GTR 23 and CMS GT 603 A X GTR 95). Out of 30 hybrids, 6 hybrids exhibited (Table 2) significant positive standard heterosis for 100-seed weight, with a range varying from -4.02 (CMS GT 33 A x GTR 8) to 8.92 per cent (CMS GT 302 A x GTR 8). The results were found agreement with Chandirakala and Raveendran (2002), Yadav and Singh (2004), Aher *et al.*, (2006) and Baskaran and Muthiah (2006) for number of seed per pod and 100-seed weight.

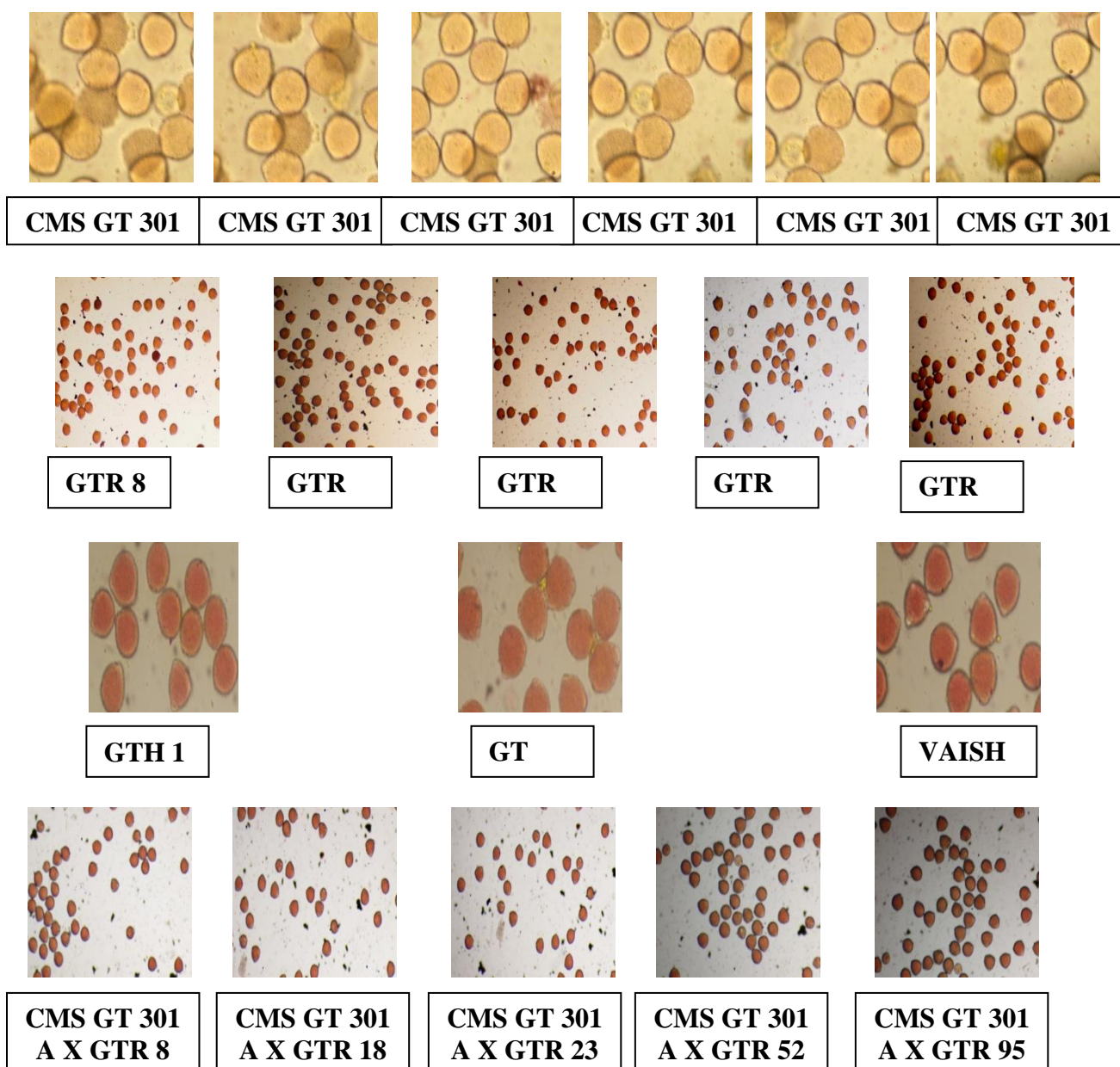
For pod length, results revealed (Table 2) that twelve hybrids significant positive standard heterosis. The standard heterosis for this trait ranged from -6.64 (CMS GT 301 A x GTR 18) to 23.67 per cent (CMS GT 288 A x GTR 95). The results were similar with the findings of Thiruvengadam and Muthiaha (2012) and Patel and Tikka (2014).

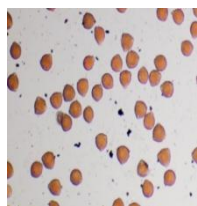
Table.1 Analysis of variance showing mean sum of squares for different characters in pigeonpea

Parents	d.f.	Days to flowering	Days to maturity	Plant height (cm)	Number of branches per plant	Number of pods per plant	Number of seeds per pod	Pod length (cm)
Replication	2	1.00	0.58	91.57	0.48	155.05	0.015	0.10
Parents	10	1903.85**	422.92**	1143.53**	11.80**	902.35**	0.38**	1.22**
Lines	5	608.36**	486.10**	539.30**	0.91	1647.27**	0.43**	1.77**
Testers	4	37.56**	243.76**	759.46**	16.15**	127.42	0.39**	0.03
Lines vs Testers	1	1333.30**	823.65**	727.45*	48.89**	277.34**	0.09**	3.26*
Parents vs Hybrids	1	198.78**	456.87**	12844.06**	1.66	280331.20**	0.11	2.90**
Hybrids	29	329.75**	258.22**	727.45**	3.72	4430.83**	0.35	0.33**
Error	80	6.33	15.37	134.75	0.45	255.49	0.05	0.08

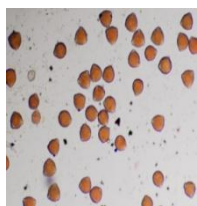
Parents	d.f	100 seed weight (g)	Seed yield per plant (g)	Harvest index (%)	Total protein content (%)	Biological yield (g)	Leaf area (cm ²)
Replication	2	0.29	50.92	7.55	0.08	52.55	13869.79**
Parents	10	0.60**	195.72**	33.91**	0.19	1903.85**	699443.80**
Lines	5	0.79**	67.29	28.31**	0.76**	878.27	744653.10**
Testers	4	0.50**	119.76*	25.65**	3.38**	2614.74**	777319.30**
Lines vs Testers	1	0.00061	1141.70**	94.96*	1.66*	4188.18**	161892.00**
Parents vs Hybrids	1	1.50**	48053.65**	265.24**	0.37	593217.10**	1428014.00**
Hybrids	29	0.27**	1458.15**	32.11**	1.09	18964.78**	366767.80**
Error	80	0.13	36.98	5.43	0.18	517.08	18290.00

Fig.1 Pollens as captured after staining with 2% aceto-carmin solution in CMS lines, Restorer lines, standard checks and F₁ hybrids

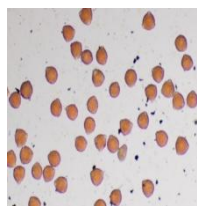




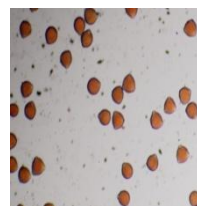
**CMS GT 302
A X GTR 8**



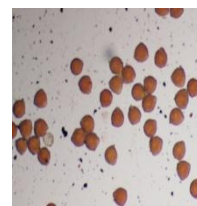
**CMS GT 302
A X GTR 18**



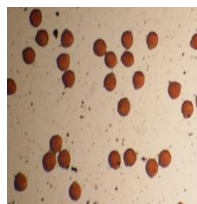
**CMS GT 302
A X GTR 23**



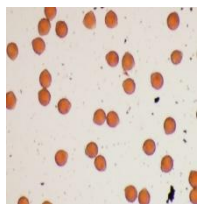
**CMS GT 302
A X GTR 52**



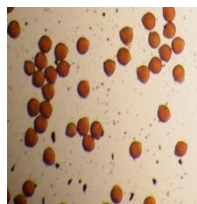
**CMS GT 302
A X GTR 95**



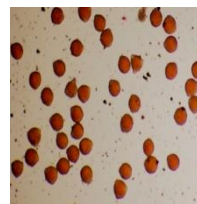
**CMS GT 33
A X GTR 8**



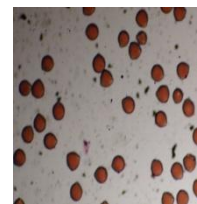
**CMS GT 33
A X GTR 18**



**CMS GT 33
A X GTR 23**



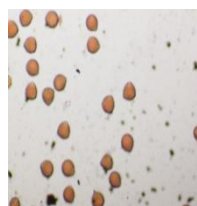
**CMS GT 33
A X GTR 52**



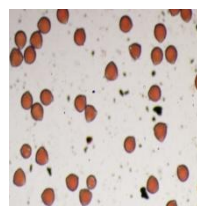
**CMS GT 33
A X GTR 95**



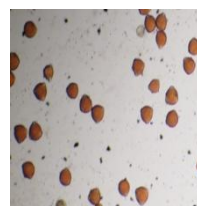
**CMS GT 288
A X GTR 8**



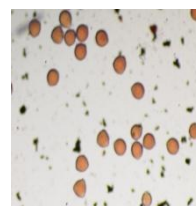
**CMS GT 288
A X GTR 18**



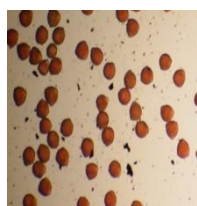
**CMS GT 288
A X GTR 23**



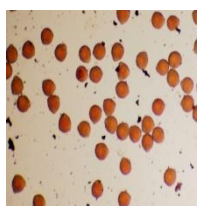
**CMS GT 288
A X GTR 52**



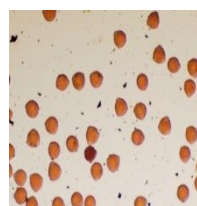
**CMS GT 288
A X GTR 95**



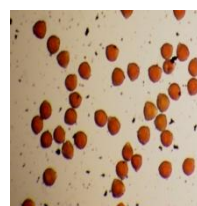
**CMS GT 601
A X GTR 8**



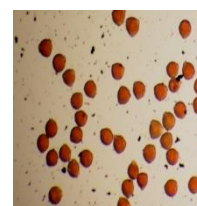
**CMS GT 601
A X GTR 18**



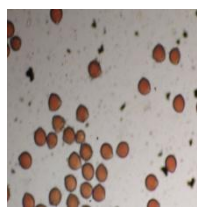
**CMS GT 601
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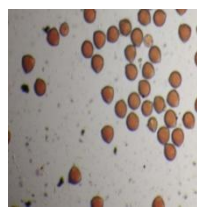
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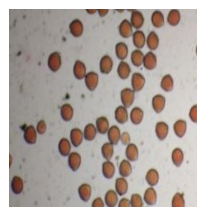
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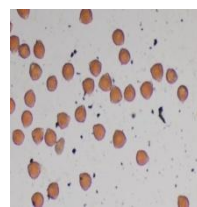
**CMS GT 603
A X GTR 8**



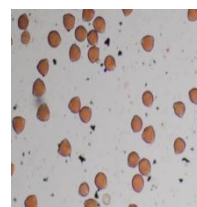
**CMS GT 603
A X GTR 18**



**CMS GT 603
A X GTR 23**



**CMS GT 603
A X GTR 52**



**CMS GT 603
A X GTR 95**

Table.2 Estimates of standard heterosis (over GTH 1) for different traits

Sr. No	Hybrids	DF	DM	PH	BPP	PP	SPP	PL
		SC	SC	SC	SC	SC	SC	SC
1.	CMS GT 301 A X GTR 8	20.42**	14.84**	-0.68	-11.92	-15.91**	-22.52**	-3.10
2.	CMS GT 301 A X GTR 18	22.18**	18.19**	10.00	-8.59	-6.11	-24.21**	-6.64
3.	CMS GT 301 A X GTR 23	20.42**	14.60**	5.95	19.62**	-11.42*	-14.53**	-5.97
4.	CMS GT 301 A X GTR 52	20.07**	16.27**	6.88	-13.72	-36.00**	1.69	-3.54
5.	CMS GT 301 A X GTR 95	16.54**	16.51**	19.35**	27.31**	15.97**	-4.84	-0.22
6.	CMS GT 302 A X GTR 8	9.51**	6.94*	6.88	10.26	0.12	-12.83**	-3.98
7.	CMS GT 302 A X GTR 18	15.14**	14.35**	13.77*	-13.72*	-13.98**	1.69	16.15**
8.	CMS GT 302 A X GTR 23	12.68**	3.83	13.40*	-16.28**	-8.49	-12.83**	2.43
9.	CMS GT 302 A X GTR 52	10.56**	10.53**	11.76	0.00	-13.17**	0.00	11.73*
10.	CMS GT 302 A X GTR 95	11.97**	11.01**	10.70	9.36	-13.83**	-3.15	14.38**
11.	CMS GT 33 A X GTR 8	-10.21**	-1.44	-2.89	-13.72*	-33.98**	-15.98**	10.84*
12.	CMS GT 33 A X GTR 18	-4.58*	3.11	10.51	-21.41**	4.33	-19.37**	-2.43
13.	CMS GT 33 A X GTR 23	-8.45**	-3.59	8.74	9.36	-14.32**	-3.15	2.21
14.	CMS GT 33 A X GTR 52	-11.62**	-2.15	-4.47	-14.49*	-24.05**	-17.68**	11.06*
15.	CMS GT 33 A X GTR 95	-13.03**	-2.87	10.14	15.77*	-6.42	1.69	2.21
16.	CMS GT 288 A X GTR 8	-1.42	3.59	3.77	-19.62**	-11.19*	0.00	13.50**
17.	CMS GT 288 A X GTR 18	1.06	8.61**	8.83	-11.15	-3.10	-17.68**	6.86
18.	CMS GT 288 A X GTR 23	0.00	9.09**	0.37	17.95*	-27.50**	1.69	9.07
19.	CMS GT 288 A X GTR 52	-0.36	8.38**	10.88	-11.92	-15.18**	0.00	11.50*
20.	CMS GT 288 A X GTR 95	0.00	3.35	19.26**	1.67	12.38*	-7.99	23.67**
21.	CMS GT 601 A X GTR 8	17.96**	13.64**	5.72	-10.26	-15.90**	-7.99	11.28*
22.	CMS GT 601 A X GTR 18	17.96**	13.88**	12.46	-15.38*	-4.08	-3.15	6.42
23.	CMS GT 601 A X GTR 23	19.36**	18.90**	7.67	-7.69	-21.64**	-7.99	3.54
24.	CMS GT 601 A X GTR 52	14.79**	13.40**	9.53	-21.41**	-0.91	-3.15	7.30
25.	CMS GT 601 A X GTR 95	19.71**	14.12**	18.98**	-5.13	10.49*	-14.53**	7.74
26.	CMS GT 603 A X GTR 8	16.19**	12.92**	28.98**	-1.67	-0.56	-14.53**	10.40*
27.	CMS GT 603 A X GTR 18	15.49**	16.03**	21.53**	-16.28*	-6.88	1.69	15.93**
28.	CMS GT 603 A X GTR 23	19.36**	16.99**	35.95**	-13.72*	15.24**	1.69	11.50*
29.	CMS GT 603 A X GTR 52	14.08**	14.12**	41.76**	12.82	15.51**	-12.83**	6.19
30.	CMS GT 603 A X GTR 95	16.19**	13.40**	31.77**	20.13**	10.09*	1.69	10.18
S.Em \pm		2.054	3.20	9.478	0.5492	13.05	0.1585	0.2309
No. of crosses showing significant desirable heterosis		5	0	0	5	6	0	12

Sr. No.	Hybrids	TSW	SY	HI	PC	BY	LA
		SC	SC	SC	SC	SC	SC
1.	CMS GT 301 A X GTR 8	2.54	-11.69	-11.91	-7.68**	0.92	0.92
2.	CMS GT 301 A X GTR 18	3.74	9.55	-23.25**	0.15	36.39**	36.39**
3.	CMS GT 301 A X GTR 23	-0.99	-9.09	-23.68**	-6.99**	30.11**	30.11**
4.	CMS GT 301 A X GTR 52	-0.46	-27.29**	-39.23**	-4.11*	31.93**	31.93**
5.	CMS GT 301 A X GTR 95	3.70	28.61**	-30.32**	-8.77**	43.59**	43.59**
6.	CMS GT 302 A X GTR 8	8.92**	33.39**	5.27	-4.46**	29.30**	29.30**
7.	CMS GT 302 A X GTR 18	-0.67	-3.45	-40.39**	-9.02**	40.44**	40.44**
8.	CMS GT 302 A X GTR 23	4.51	3.48	-19.78**	-7.83**	38.46**	38.46**
9.	CMS GT 302 A X GTR 52	-0.07	7.81	-22.82**	-8.67**	33.36**	33.36**
10.	CMS GT 302 A X GTR 95	-0.35	3.91	-21.25**	-6.24**	36.11**	36.11**
11.	CMS GT 33 A X GTR 8	-4.02	-16.45*	-21.41**	2.03	-9.77	-9.77
12.	CMS GT 33 A X GTR 18	3.28	47.69**	3.94	0.94	33.24**	33.24**
13.	CMS GT 33 A X GTR 23	-1.59	-4.32	-18.45**	-8.52**	31.42**	31.42**
14.	CMS GT 33 A X GTR 52	7.05*	-17.75**	-21.35**	-7.93**	-6.65	-6.65
15.	CMS GT 33 A X GTR 95	1.69	27.76**	-8.77	-6.84**	32.83**	32.83**
16.	CMS GT 288 A X GTR 8	0.00	-3.02	-19.91**	-7.43**	-4.41	-4.41
17.	CMS GT 288 A X GTR 18	2.50	8.68	-19.51**	-6.24**	29.60**	29.60**
18.	CMS GT 288 A X GTR 23	-0.78	-21.65**	-18.85**	-8.82**	4.32	4.32
19.	CMS GT 288 A X GTR 52	8.43**	-3.02	-27.45**	-7.58**	35.19**	35.19**
20.	CMS GT 288 A X GTR 95	-1.52	57.22**	-16.81**	-7.04**	39.79**	39.79**
21.	CMS GT 601 A X GTR 8	5.01	-13.85*	-27.52**	-8.57**	28.02**	28.02**
22.	CMS GT 601 A X GTR 18	-0.07	15.18*	-21.65**	-4.91**	33.74**	33.74**
23.	CMS GT 601 A X GTR 23	-2.19	-24.25**	-39.53**	-3.87*	23.09**	23.09**
24.	CMS GT 601 A X GTR 52	-0.11	37.30**	0.40	-4.06*	28.31**	28.31**
25.	CMS GT 601 A X GTR 95	4.20	49.41**	-8.81	-9.12**	44.95**	44.95**
26.	CMS GT 603 A X GTR 8	4.44	46.81**	-20.88**	-8.33**	56.38**	56.38**
27.	CMS GT 603 A X GTR 18	0.35	21.68**	-27.95**	-7.93**	49.04**	49.04**
28.	CMS GT 603 A X GTR 23	-0.95	64.59**	-19.21**	-9.56**	57.74**	57.74**
29.	CMS GT 603 A X GTR 52	-0.21	69.79**	-17.78**	-7.93**	62.93**	62.93**
30.	CMS GT 603 A X GTR 95	-0.49	53.76**	-19.45**	-7.53**	55.21**	55.21**
S.Em +		0.3008	4.965	1.901	0.3533	18.56	110.42
No. of crosses showing significant desirable heterosis		3	13	0	0	9	25

Table.3 Best ten high yielding hybrids with heterosis (%) over standard check (GTH 1) and showed high fertility restoration (%)

Sr. No.	Ten best hybrids on the basis of heterosis (%) of seed yield	Mean seed yield per plant (g)	Heterosis (%)	Significant standard heterosis for component traits in desired direction	Pollen Fertility Restoration (%)
			SC (GTH 1)		
1.	CMS GT 603 A X GTR 52	130.60	69.79**	PP, BY, LA	100.00
2.	CMS GT 603 A X GTR 23	126.60	64.59**	PP, PL, BY, LA	98.22
3.	CMS GT 288 A X GTR 95	120.93	57.22**	PP, PL, BY, LA	100.00
4.	CMS GT 603 A X GTR 95	118.27	53.76**	PP, BY, LA, BPP	94.42
5.	CMS GT 601 A X GTR 95	114.93	49.41**	PP, BY, LA	100.00
6.	CMS GT 33 A X GTR 18	113.60	47.69**	LA, PC	100.00
7.	CMS GT 603 A X GTR 8	112.93	46.81**	LA, PL	100.00
8.	CMS GT 601 A X GTR 52	105.61	37.30**	LA	95.302
9.	CMS GT 302 A X GTR 8	102.60	33.39**	LA, TSW	100.00
10.	CMS GT 301 A X GTR 95	98.93	28.61**	PP, LA, BY, BPP	94.33

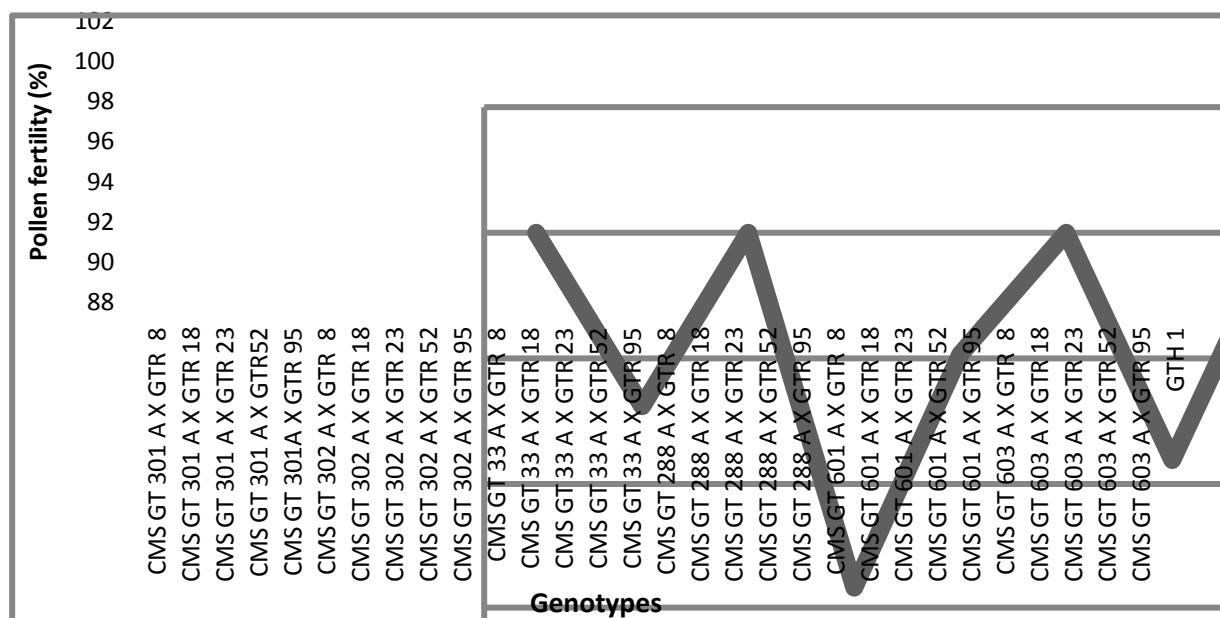
Table.4 Pollen fertility status recordd for female, male parent, F₁ hybrids & standard checks in pigeonpea

Sr. No.	Genotype	R I*		R II*		R III*		Total		Total pollen observed	Plant fertility\$	Pollen Fertility (%)
		Sterile	Fertile	Sterile	Fertile	Sterile	Fertile	sterile	Fertile			
1	P1 : GT 301A	69.40	-	74.40	-	58.20	-	202.00	-	202.00	S	0.00
2	P2 : GT 302A	85.20	-	76.40	-	62.00	-	223.60	-	223.60	S	0.00
3	P3 : GT 33A	81.60	-	92.00	-	73.20	-	246.80	-	246.80	S	0.00
4	P4 : GT 288A	52.00	-	63.20	-	86.40	-	201.60	-	201.60	S	0.00
5	P5 : GT 601A	76.20	-	58.80	-	67.60	-	202.60	-	202.60	S	0.00
6	P6 : GT 603A	91.20	-	75.20	-	83.80	-	250.20	-	250.20	S	0.00
7	P7 : GTR 8	-	104.60	-	86.20	-	112.00	-	302.80	302.80	F	100.00
8	P8 : GTR 18	-	96.20	-	110.20	-	78.40	-	284.80	284.80	F	100.00
9	P9 : GTH 23	-	131.20	-	108.60	-	120.00	-	359.80	359.80	F	100.00
10	P10:GTH 52	4.00	87.80	-	111.40	2.00	124.40	6.0	323.60	329.60	F	98.18
11	P11:GTH 95	1.20	140.00	3.20	122.00	1.40	107.20	5.80	369.20	375.00	F	98.45
12	CMS GT 301 A X GTR 8	-	165.60	-	98.20	-	130.00	-	393.80	393.80	F	100.00
13	CMS GT 301 A X GTR 18	3.00	156.20	2.00	87.00	5.40	122.60	10.40	365.80	376.20	F	97.24
14	CMS GT 301 A X GTR 23	-	145.20	-	101.40	-	79.20	-	325.80	325.80	F	100.00
15	CMS GT 301 A X GTR52	10.20	136.40	8.60	87.20	3.20	142.40	22.00	366.00	388.00	F	94.33
16	CMS GT 301A X GTR 95	2.40	134.00	1.40	93.20	3.00	117.20	6.80	344.40	351.20	F	98.06
17	CMS GT 302 A X GTR 8	-	122.00	-	146.40	-	109.40	-	377.80	377.80	F	100.00
18	CMS GT 302 A X GTR 18	4.40	162.20	6.20	132.40	5.00	118.20	15.60	412.80	428.40	F	96.36
19	CMS GT 302 A X GTR 23	-	96.80	-	89.00	-	132.00	-	317.80	317.80	F	100.00
20	CMS GT 302 A X GTR 52	6.20	158.60	9.40	142.60	12.00	126.40	27.60	427.60	455.20	F	93.94
21	CMS GT 302 A X GTR 95	11.40	123.20	14.00	146.80	7.20	116.00	32.60	386.00	418.60	F	92.21

22	CMS GT 33 A X GTR 8	-	98.20	-	133.20	-	153.20	-	384.60	384.60	F	100.00
23	CMS GT 33 A X GTR 18	-	148.40	-	88.20	-	128.40	-	365.00	365.00	F	100.00
24	CMS GT 33 A X GTR 23	3.60	137.00	1.40	130.00	3.40	143.00	8.40	410.00	418.40	F	97.99
25	CMS GT 33 A X GTR 52	1.20	117.20	4.00	144.60	2.00	96.40	7.20	358.20	365.40	F	98.03
26	CMS GT 33 A X GTR 95	-	98.20	-	123.40	-	156.00	-	377.60	377.60	F	100.00
27	CMS GT 288 A X GTR 8	-	167.60	-	128.20	-	107.20	-	403.00	403.00	F	100.00
28	CMS GT 288 A X GTR 18	2.00	146.40	1.60	118.20	3.20	113.40	6.80	378.00	384.80	F	98.23
29	CMS GT 288 A X GTR 23	-	127.00	-	138.60	-	106.20	-	371.80	371.80	F	100.00
30	CMS GT 288 A X GTR 52	6.20	132.40	3.20	105.40	9.20	128.40	18.60	366.20	384.80	F	95.17
31	CMS GT 288 A X GTR 95	-	112.20	-	153.00	-	147.80	-	413.00	413.00	F	100.00
32	CMS GT 601 A X GTR 8	3.40	144.60	1.20	123.20	2.40	74.60	7.00	342.40	349.40	F	98.00
33	CMS GT 601 A X GTR 18	-	163.00	-	116.40	-	135.00	-	414.40	414.40	F	100.00
34	CMS GT 601 A X GTR 23	4.20	78.40	3.00	148.80	2.00	124.20	9.20	351.40	360.60	F	97.45
35	CMS GT 601 A X GTR 52	5.00	94.40	8.40	126.40	4.20	137.40	17.60	358.20	375.80	F	95.32
36	CMS GT 601 A X GTR 95	-	138.20	-	115.00	-	130.00	-	383.20	383.20	F	100.00
37	CMS GT 603 A X GTR 8	-	156.60	-	121.20	-	103.60	-	381.40	381.40	F	100.00
38	CMS GT 603 A X GTR 18	2.40	149.60	1.20	137.20	5.60	125.00	9.20	411.80	421.00	F	97.81
39	CMS GT 603 A X GTR 23	3.20	89.20	2.00	148.20	1.40	127.00	6.60	364.40	371.00	F	98.22
40	CMS GT 603 A X GTR 52	-	107.60	-	105.00	-	135.20	-	347.80	347.80	F	100.00
41	CMS GT 603 A X GTR 95	11.20	136.00	7.40	162.40	5.00	101.20	23.60	399.60	423.20	F	94.42
42	GTH 1	6.40	96.40	1.00	103.80	2.20	115.60	9.60	315.80	325.40	F	97.05
43	GT 101	3.00	121.40	2.60	89.60	1.80	106.40	7.40	317.40	324.80	F	97.72
44	VAISHALI	1.00	78.20	4.00	135.00	2.20	109.20	7.20	322.40	329.60	F	97.82

* = Average of observations of five plants; \$ = Plant sterility: S = sterile and F= Fertile

Fig.2 The graph showing pollen fertility status of different CMS-based hybrids and check of pigeonpea



Harvest index indirectly influences the seed yield through partitioning photosynthates in source and sink. None of the hybrids reported (Table 2) significant and positive standard heterosis. It ranged from -40.39 (CMS GT 302 A X GTR 18) to 5.27 per cent (CMS GT 302 A X GTR 8). The results were in agreement with Patel *et al.*, (1991), Aghav *et al.*, (1997), Phad *et al.*, (2009) and Gupta *et al.*, (2011) for harvest index.

None of the hybrids exhibited significant positive heterosis against GTH 1 for protein content. The standard heterosis ranged from -9.56 (CMS GT 603 A X GTR 23) to 2.03 per cent (CMS GT 33 A X GTR 8). For biological yield per plant, nine hybrids exhibited significant and positive standard heterosis, that ranged from -34.12 (CMS GT 288 A X GTR 23) to 40.57 per cent (CMS GT 603 A X GTR 52) for GTH 1. These results were in close agreement with Baskaran and Muthiah (2006) and Patel and Tikka (2008) for protein content and biological yield per plant.

The growth of plant in term of mass will depend on how carbon is partitioned among new leaf area, leaf mass, root mass. The perusal of the results revealed (Table 2) that twenty five hybrids exhibited significant and positive standard heterosis. The range of standard heterosis was from -9.77 (CMS GT 33 A X GTR 8) to 62.93 per cent (CMS GT 603 A X GTR 52).

To confirm pollen fertility restoration in hybrids, the differences observed due to pollen fertility as tested pollen grains (with 2% acetocarmine solution) of parents ('A' lines and male parents), hybrids and standard checks are given in Table 3. The male sterile lines (P1) exhibited (100 per cent) pollen sterility. The pollen fertility in fertility restorer lines (P2) varied from 98 to 100 per cent. The F1 generations of all the crosses exhibited pollen fertility restoration varying from 92 to 100 percent. Standard checks GTH 1, GTH 101 and VAISHALI exhibited 97, 98 and 97 per cent pollen fertility respectively. The pollen fertility was observed under light

microscope and on the basis of its staining properties, pollen grains were categorized as fertile (deep to light red color) and sterile (shriveled, hyaline, transparent) of different parents (P1 and P2), all the F1 crosses and standard checks given in Fig. 1 and data shown in FIG. 2 for fertility status of hybrids. The anthers of male sterile lines, restorer lines, F1 hybrids and standard checks were observed on visual basis for pollen fertility status, indicated male sterile lines had translucent type anthers whereas fertile lines (male parents, hybrids and checks) had normal pollen shedding in anthers. The results of present findings were in agreement to findings of Saxena and Kumar (2003), Chauhan *et al.*, (2004), Mallikarjuna and Saxena (2005), Nadrajan *et al.*, (2008), Kalaimagal *et al.*, (2012), Saxena *et al.*, (2013), Saxena *et al.*, (2014) and Chaudhary *et al.*, (2015).

From the study of heterosis and fertility restoration, it was concluded that to evaluate the restoration of fertility, Pollen fertility (%) and amount of viable pollens produced by particular hybrid are an important characters which is a basic need for the successful production of high yielding CMS-based hybrids of pigeonpea. Due to success breeding of a stable CMS system and quality fertility restorers, we can standardized seed production technology.

The best cross combinations (Table 4) in order of merit seed yield and other yield components were CMS GT 603 A X GTR 52 (69.79 %), CMS GT 603 A X GTR 23 (64.59 %), CMS GT 288 A X GTR 95 (57.22 %) and CMS GT 603 A X GTR 95 (53.76 %), CMS GT 601 A X GTR 95 (49.41 %), CMS GT 33 A X GTR 18 (47.69 %), CMS GT 603 A X GTR 8 (46.81 %), CMS GT 601 A X GTR 52 (37.30 %), CMS GT 302 A X GTR 8 (33.39 %) and CMS GT 301 A X GTR 95 (28.61 %). These hybrids had high per se performance

for grain yield, more standard heterosis with one or more its contributing traits viz. number of pods per plant, biological yield, leaf area and harvest index and having high pollen fertility status suggested that these hybrids can be directly exploited commercially after evaluating their performance in wide range of environment. These crosses can also be used to throw-off transgressive segregants in segregating generations for the improvement of yield and specific yield attributing traits.

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