

Original Research Article

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Utilization of Different Height Variant Alleles in CGMS System Derived from A₂ Cytoplasm in Pigeonpea [*Cajanus cajan* (L.) Millsp]

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ABSTRACT

CGMS based hybrid breeding was found more reliable to give productive hybrids in pigeonpea but need some expansion. Therefore, in the present research, cytoplasmic male sterility (CMS) source from line AK-120-1A (A₂ cytoplasm) was diversified into different height variant lines. These four height variant lines were combined with cytoplasmic male sterility through repeated backcrossing. Anthers of new CMS lines were white translucent colored that strongly correlated with complete male sterility without pollen grains. CMS lines showed difference in height, possibly controlled by different alleles of a gene. Two lines, Dwarf 45 cm and Dwarf 60 cm were early in the flowering and maturity. During the evaluation, Dwarf 45 cm and Dwarf 90 cm CMS lines found good for heterosis and for fertility restoration in combination with twelve restorer lines. Two hybrids Dwarf 45 cm x AKPR-344 and Dwarf 45 cm x AKPR-325 were found significant over the checks PKV-TARA and AKT-8811 for yield traits and found completely fertile. These dwarf lines showed good performance in hybrid combinations showing the prospects for future hybrid breeding.

Keywords

CGMS, Backcrossing, Height Variants, Heterosis, Fertility restoration.

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Introduction

Pigeonpea is one of the important grain legumes in Indian sub-continent, which is a good source of protein. Besides that, pigeonpea is an often cross-pollinated crop (upto 70% cross-pollination) (Saxena *et al.*, 1990), and the ability of cross pollination will add some benefit in the hybrid seed production. The productivity of pigeonpea was stagnated around 0.6-0.7 t/ha from the last few decades (Saxena *et al.*, 2005). To break such productivity barriers, hybrid breeding will be option to enhance yield and productivity. For developing the hybrids,

male sterile line controlled by nuclear gene was identified (Reddy *et al.*, 1978). So far different ms genes were identified and utilized in hybrid breeding. Based on GMS system six hybrids were released so far with 30-40% higher yield over present cultivars. Unfortunately, hybrids were not popularized due to rouging problem associated with a GMS system which increases cost of seed production and impurity in hybrid seed. While searching for another male sterility source associated with the cytoplasmic genome in pigeonpea, stable CMS lines were developed

from *C. Scarabaeoides* (Tikka *et al.*, 1997) and *C. cajanifoliosus* (Saxena *et al.*, 2005) species. From these CMS lines three hybrids (GTH-1, ICPH-2671 and ICPH-2740) were developed and released so far. CGMS hybrid breeding technique is popular in different crops due to stable male sterility and ease of maintenance of purity of CMS lines. In spite of these advantages, very few hybrids were developed and released in pigeonpea using the CGMS system.

Different CMS lines were developed from available sources in pigeonpea, but these are few in numbers and most of them have similar plant type with narrow genetic variability. These CMS lines are difficult to differentiate morphologically from restorers and hybrids.

There is a need to diversify CMS lines and restorer lines in different genetic background to create some variation and utilize them in hybrid breeding. Present research efforts were made to enhance the variability in CMS lines through conversion of dwarf four lines viz., Dwarf 30 cm, Dwarf 45 cm, Dwarf 60 cm and Dwarf 90 cm into cytoplasmic male sterility. Variation in the height of the parents will help in identifying the out crossed seeds from CMS and selfed-seeds from the hybrid populations. In present study new height variant CMS lines were tested for fertility restoration gene and for heterosis over checks.

Materials and Methods

Diversification of CMS

In the diversification process, CMS line AK-120-1A source of A₂ cytoplasm was crossed with four dwarf lines viz., Dwarf 30 cm, Dwarf 45 cm, Dwarf 60 cm and Dwarf 90 cm (Fig. 1) during rainy season, 2006. These height variants were showed marked differences in height of stem implying that

these lines may have different alleles of the same gene from *Cajanus cajan* (Fig. 1). Sterile plants were identified in F₁ generations of each cross during 2007-08 based on anther color, pollen fertility [tested using 1% Potassium Iodine Iodide (KII) stain] and anther dehiscence. Sterile plants with translucent anthers were selected for further backcrossing.

For testing male sterility, anthers were taken randomly from 3-5 flowers from each plant at 50% flowering and crushed on slide with 1% KII stain and examined under simple microscope at 45x. Completely stained (deep blue colored) pollen grains were considered as fertile (Fig. 2c) while, unstained pollen grains (Fig. 2b) and anthers devoid of pollen grains (Fig. 2a) were considered as sterile.

In BC₁F₁ generation sterile plants similar to recurrent parent were selected and sib mated with recurrent parents during backcrossing. In the similar way sterile plants were identified and backcrossed with recurrent parents. Dwarf plants were selected at the advent of flowering in each line during backcrossing. Schematic diagram of backcrossing programme for diversification of CMS into dwarf lines used in the research plan is given in Figure 3.

Characterization of CMS lines

Characterizations of these new CMS lines was done from BC₃F₁ generation after getting complete sterility and homogeneity between A and B-line of respective dwarf lines. Observations were recorded on sterility, seed color, plant growth habitat, flower color, streaks on standard petal, pod streaks, days to 50% flowering, plant height and number of primary branches. Evaluation of yield and yield contributing traits was done in the rainy season, 2011. Observations were recorded in B-lines of respective CMS lines on a number

of days to maturity, pods per plant, grains per pod, 100 seed weight (g) and grain yield per plant (g).

Evaluation of hybrids: Fertility restoration and heterosis

In the Kharif season 2010, 48 hybrids were developed by using 12 restorer lines AKPR-8, AKPR-12, AKPR-178 (E), AKPR-178(M), AKPR-210, AKPR-249, AKPR-292, AKPR-319, AKPR-325, AKPR-344, AKPR-359 and AKPR-364 and 4 dwarf CMS lines. These hybrids were tested for fertility restoration and for different yield traits in the rainy season, 2011. These 48 hybrids along with 12 restorer lines, 4 maintainer lines and two high yielding checks PKV-TARA and AKT-8811 were evaluated in a randomized complete block design (RCBD) with three replications during the kharif season, 2011. Data were recorded on eight yield and yield contributing traits. Hybrids were also evaluated for fertility restoration with on pollen fertility, anther color and anther dehiscence. Fertility of pollen grains from every F_1 plants of respective hybrid was tested by using 1% potassium iodine iodide (KII) stains.

Results and Discussion

Diversification of CMS

In the present study, source of cytoplasmic male sterile was used from AK-120-1A CMS line which was developed from A_2 cytoplasmic (*C. scarabaeoides*) source. Line AK-120-1A had white translucent anthers with tall plant stature. These white translucent anthers were found with devoid of pollen grains and showed complete male sterility.

During 2006-07, four dwarf stature lines (Fig. 1) were crossed with AK-120-1A CMS line and tall plants with male sterility were observed in F_1 generation. Three crosses

involving Dwarf 30 cm, Dwarf 45 cm and Dwarf 60 cm showed 100% male sterility whereas Dwarf 90 cm line showed 94.74% sterility in the F_1 generation. Maximum sterility in F_1 reveals that the absence of dominant fertility restorer gene in the nucleus of the four recurrent parents, hence these parents were used as maintainer of dwarf CMS line. From F_1 populations, sterile plants with translucent anther were selected in each cross and backcrossed with respective dwarf lines. In BC_1F_1 generation plants were segregated in two categories, tall plants and dwarf plants approximately in the ratio 1:1. Results of backcrossing of four parents are given below.

In case of Dwarf 30 cm, 87.09% plants were sterile in BC_1F_1 generation among which 14 plants were dwarf. Some of the plants exhibited yellow shriveled anthers were rejected. In subsequent generations (BC_2F_1 , BC_3F_1 , BC_4F_1 , BC_5F_1) of backcrossing, 100% sterility with white translucent anthers were observed. Morphology of the recurrent parent Dwarf 30 cm was recovered completely in the newly developed CMS lines. The line is designated as Dwarf 30 cm CMS.

After getting complete sterility in the F_1 generation of Dwarf 45 cm, number of sterile plants was reduced to 62.06% in BC_1F_1 generation. Among the sterile plants, 13 were dwarf and remaining plants with low pollen fertility (<20%).

Sterile dwarf plants were sib mated with recurrent parent Dwarf 45 cm. Later in BC_2F_1 generation, 93.02% plants were male sterile and dwarf. Further in BC_3F_1 , BC_4F_1 and BC_5F_1 generations, 100% plants were sterile and similar to the parent Dwarf 45 was recovered. In the backcrossing of Dwarf 60 cm, early generations BC_1F_1 , BC_2F_1 and BC_3F_1 showed low male sterility percent (63.63%, 46.15%, and 86.11% respectively)

in BC₄F₁ generation 100% sterile plants were recovered. At the end of BC₅F₁ generation, a new male sterile line with the morphology of the Dwarf 60 cm line was developed. While backcrossing another line Dwarf 90 cm showed 94.74% sterility in F₁ generation and in next BC₁F₁ generation 15 dwarf plants were completely male sterile. These dwarf plants were backcrossed with Dwarf 90 cm. Plant type of Dwarf 90 cm and male sterility was recovered in BC₃F₁ and later generations (Table 1).

In the present research, male sterility combined successfully with the dwarfing genes and developed four new CMS lines with dwarf stature. It was observed that the white translucent anther is an important trait for developing complete sterile lines in diversification of male sterility source especially from A₂ cytoplasm.

Characterization of new CMS lines

All the four new CMS lines (A-lines) showed similarity with respect to B-lines (recurrent parent) except for gene of male fertility. Sterile plants of four new CMS lines exhibited white translucent anthers devoid of pollen grains whereas B-lines had yellow color anthers with good pollen dehiscence.

Each line had characteristic height, 37.8±1.32 cm for Dwarf 30 cm, 53.7±1.92 cm for Dwarf 45 cm, 67.9±2.37 cm for Dwarf 60 cm and 118.71±4.81 cm for Dwarf 90 cm. Three lines (Dwarf 30 cm, Dwarf 45 cm and Dwarf 60cm) exhibited semi-spreading branches whereas Dwarf 90 cm exhibited spreading type branching pattern and indeterminate growth habit.

For seed color, Dwarf 45 cm exhibited white seed whereas others were brown colored. Stem pigmentations were absent in all the four CMS lines and respective maintainer

lines. Streaks were present sparsely on standard petals of flower in Dwarf 60 cm line, whereas these streaks were absent in other three lines Dwarf 30 cm, Dwarf 45 cm and Dwarf 90 cm.

While observing the yield parameters, highest pod bearing (95.15±0.41) and seed yield per plant (29.39±2.69 g) observed in Dwarf 90 cm line than the other lines. Two lines Dwarf 45 cm and Dwarf 60 cm found early in flowering and maturity, whereas Dwarf 30 cm was medium in flowering (109-110 days) and maturity (146 days). These dwarf lines will be best suited in high density planting with comparable yields with other tall lines. Data is presented in the Table 2.

Fertility restoration

Restoration of male fertility in hybrids is important which will decide the utilization of CMS lines in hybrid breeding. In the present investigation, these new CMS lines were tested for fertility restoration using 12 restorer lines (Table 3). The fertility data of 48 F₁ hybrids showed that the 12 restorers had genes for fertility and successfully restored the fertility in new CMS lines. Seventeen of the hybrids were found 100% male fertile, while remaining showed partial fertility. Range of partial fertility in the hybrids was from 62.75% to 97.92% indicating that the restorer lines have alleles for male fertility with low expression of fertility. Two restorers, AKPR-8 and AKPR-319 were found best for restoration of fertility in four CMS lines. Range of pollen fertility score in complete fertile hybrids was from 87.96±4.63% (Dwarf 90 cm x AKPR-319) to 97.16±1.23% (Dwarf 45 cm x AKPR-344). Parse pollen production may arise due to heterogeneity for fertility restoring genes within restorers (Saxena *et al.*, 2011) and also the genetic background of the genotype or effect of the micro environment (Kaul, 1988).

Table.1 Different generations of diversification and data of sterility percentage (no. of sterile plants)

Year	Generations	Recurrent Parents with percent sterility (no. of sterile plants)			
		Dwarf 30 cm	Dwarf 45 cm	Dwarf 60 cm	Dwarf 90 cm
Rainy season, 2007	F ₁	100(13 plants)	100 (19 plants)	100 (12 plants)	94.74 (18 plants)
Rainy season,2008	BC ₁ F ₁	87.09 (27 plants)	62.06 (18 plants)	63.63 (21 plants)	96.55 (28 plants)
Rainy season, 2009	BC ₂ F ₁	100 (33 plants)	93.02 (41 plants)	46.15 (18 plants)	76.67 (23 plants)
Rainy season,2010	BC ₃ F ₁	100 (57 plants)	100 (63 plants)	86.11 (31 plants)	100 (38 plants)
Rainy season,2011	BC ₄ F ₁	100 (84 plants)	100 (93 plants)	100 (53 plants)	100 (67 plants)
Rainy season, 2012	BC ₅ F ₁	100 (112 plants)	100 (135 plants)	100 (86 plants)	100 (105 plants)

Table.2 Description characters of different new A- lines and their respective maintainers recorded at Akola

Characters	Dwarf 30 cm A	Dwarf 30 cm B	Dwarf 45 cm A	Dwarf 45 cm B	Dwarf 60 cm A	Dwarf 60 cm B	Dwarf 90 cm A	Dwarf 90 cm B
Sterility %	100	-	100	-	100	-	100	-
Anther color	White Translucent	Yellow	White Translucent	Yellow	White Translucent	Yellow	White Translucent	Yellow
Flower Color	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
Standard petal streak	Absent	Absent	Absent	Absent	Sparsely present	Sparsely present	Absent	Absent
Keel Structure	Closed	Closed	Closed	Closed	Closed	Closed	Closed	Closed
Pod Streak	Present	Present	Present	Present	Present	Present	Present	Present
Seed color	Brown	Brown	White	White	Brown	Brown	Brown	Brown
Growth Habit	Determinate	Determinate	Determinate	Determinate	Determinate	Determinate	Indeterminate	Indeterminate
Branching pattern	Small Bushy	Small Bushy	Semi-spreading	Semi-spreading	Semi-spreading	Semi-spreading	Spreading	Spreading
Days to 50% flowering	110±0.65	109 ±0.82	77±0.72	75±0.54	78±0.69	77±0.82	127±0.45	125±0.27
Days to Maturity	-	146	-	128	-	132	-	172
Height (cm)	37.8±1.32	38.5±1.67	53.7±1.92	52.2±2.23	67.9±2.37	66.7±2.59	118.71±4.81	116.6±4.81
No. of Primary	6.68±2.63	6.53±2.42	7.12±1.42	6.95±1.56	8.34±1.97	8.08±1.84	10.12±2.10	9.93±2.24
No. of pods/plant	-	35.53±0.71	-	41.53±0.55	-	44.1±0.26	-	95.15±0.41
Grains per Pod	-	3.22±0.2	-	3.46±0.4	-	3.5±0.3	-	3.59±0.4
100 Seed Weight (g)	-	8.48±0.88	-	6.3±1.12	-	8.09±1.36	-	8.81±1.12
Yield per Plant (g)	-	9.42±1.34	-	9.05±1.54	-	11.9±1.36	-	29.39±2.69

Table.3 Fertility restoration and pollen fertility score of different hybrids

Restorers	Dwarf 30 cm		Dwarf 45 cm		Dwarf 60 cm		Dwarf 90 cm	
	F.R.%	P.F.S.%	F.R.%	P.F.S.%	F.R.%	P.F.S.%	F.R.%	P.F.S.%
AKPR-249	90.24	74.86±3.26	100	78.8±5.64	89.58	73.46±4.62	97.44	92.01±4.45
AKPR-178 (E)	92.31	85.26±4.12	89.58	79.98±3.45	100	92.89±6.45	62.75	55.1±4.25
AKPR-12	63.64	56.92±2.22	62.2	52.86±7.64	90	78.69±5.34	87.5	79.08±6.21
AKPR-178 (M)	88.24	83.72±6.52	80	58.12±4.32	88.1	80.12±5.26	91.89	81.73±3.45
AKPR-344	94.44	81.19±7.45	100	97.16±1.23	90.63	77.32±3.57	100	95.28±2.61
AKPR-8	100	93.43±3.56	97.92	92.53±4.15	100	95.85±3.69	100	94.68±2.87
AKPR-210	78.05	72.88±4.62	95.56	82.32±6.25	100	94.78±3.65	100	94.22±3.98
AKPR-325	94.44	84.43±2.27	84.78	72.69±5.36	100	92.01±2.22	100	91.24±4.56
AKPR-364	96.97	83.84±3.78	88.68	76.45±5.92	91.3	84.18±6.52	96.88	80.28±5.12
AKPR-319	100	94.77±4.12	100	95.32±2.78	100	94.8±3.65	100	87.96±4.63
AKPR-359	100	90.16±1.98	94.44	87.12±6.48	94.29	84.15±4.57	86.11	76.01±2.98
AKPR-292	100	92.23±2.14	85.42	71.92±5.25	92.86	83.45±6.22	75.61	60.38±3.24

Note: F.R.- Fertility restoration, P.F.S.- Pollen fertility score using KII stain, ±- range of variation in the hybrid

Table.4 *Per se* performance of top hybrids for different yield and yield contributing trait with economic heterosis

Hybrids	Days to Maturity	Height (cm)	No. of Primary	No. Of pods/plant	100 Grain Weight (g)	Yield per Plant (g)	Heterosis	
							H3a	H3b
Dwarf 45 cm X AKPR-344	135	138.63	9.93	225.95	8.58	63.26	19.41 **	65.73**
Dwarf 45 cm X AKPR-325	135	136.05	10.65	181.2	9.18	62.21	17.43 *	62.98**
Dwarf 90 cm X AKPR-292	159	157.29	8.8	192.32	8.24	56.85	7.32	48.94**
Dwarf 30 cm X AKPR-8	155	142.19	11.05	159.91	8.6	46.83	-11.6	22.69*
Dwarf 45 cm X AKPR-210	128	145.69	12.45	147.13	8.7	46.3	-12.61	21.30*
Dwarf 30 cm X AKPR-210	129	135.15	12.73	135.8	8.8	42.15	-20.44 **	10.43
Dwarf 45 cm X AKPR-364	135	133.82	13.33	141.99	8.38	42.08	-20.57 **	10.24
Dwarf 30 cm X AKPR-178 (E)	135	139.4	12	164.21	7.52	39.31	-25.80 **	2.99
Dwarf 90 cm X AKPR-178 (M)	158	151.92	9.55	121.47	8.53	38.85	-26.67 **	1.78
Dwarf 90 cm X AKPR-8	167	137.14	9.65	143.31	9.1	38.26	-27.79 **	0.24



Fig. 1: Different height variants (a) Dwarf 30 cm (b) Dwarf 45 cm (c) Dwarf 60 cm (d) Dwarf 90 cm



a) Anther without pollen grains (Sterile)



b) Unstained pollen grains (Sterile)



c) Darkly stained pollen grains (Fertile)

Fig.2 Differences in the staining of pollen grains

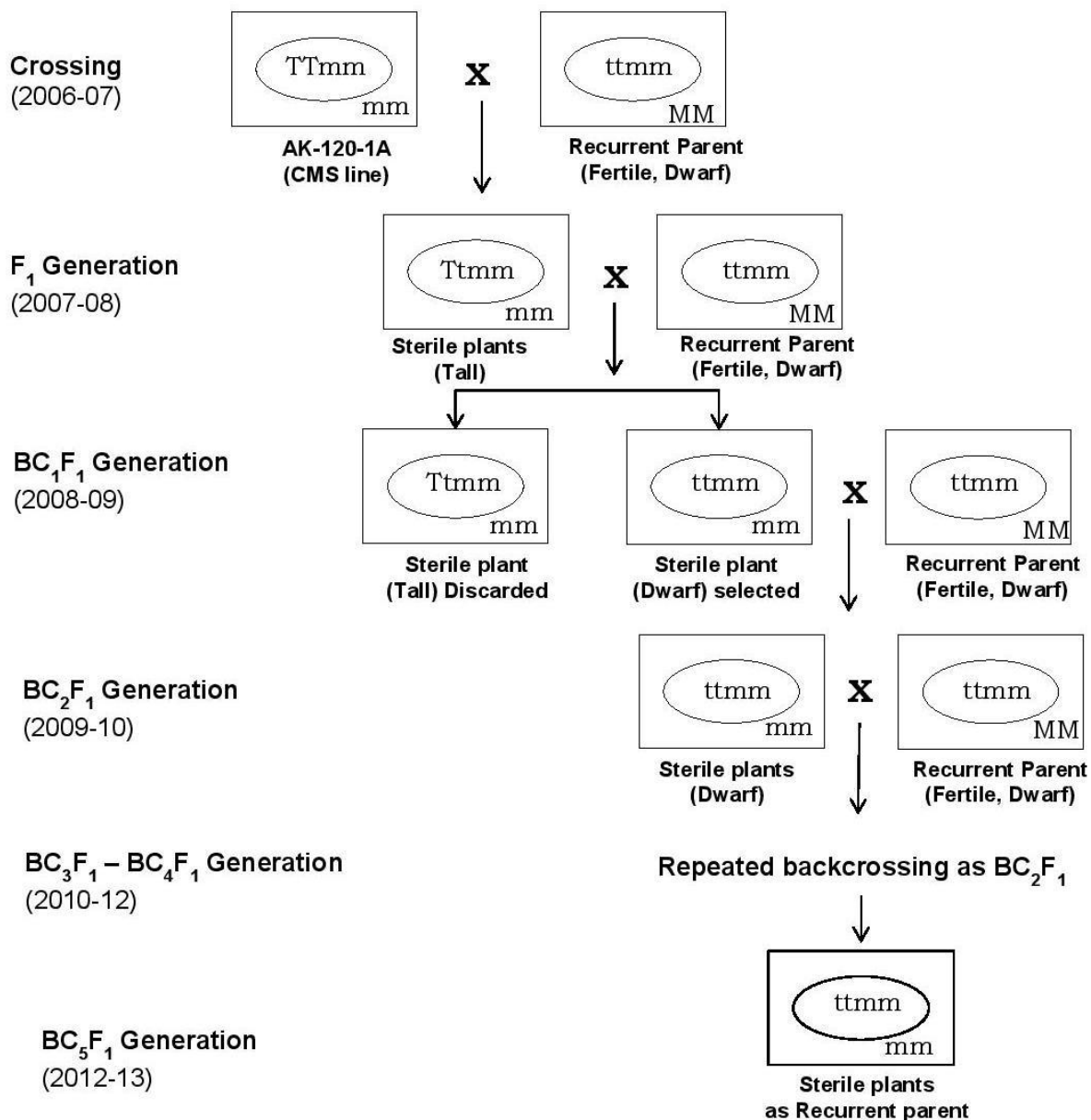


Fig.3 Schematic of crossing programme used for diversification of CMS into dwarf lines

Utilization of CMS lines in hybrid breeding

Per se performance and heterosis estimates of selected hybrids are given in the Table 4. Among these hybrids, Dwarf 45cm x AKPR-344 (63.26 g) and Dwarf 45 cm x AKPR-325 (62.21 g) showed highest per se performance for yield per plant these hybrids also showed

highest significant yield over check both the high yielding checks PKV-TARA and AKT-8811 for standard heterosis. Among the rest of the hybrids, Dwarf 90 cm x AKPR-292, Dwarf 30 cm x AKPR-8 and Dwarf 45 cm x AKPR-210 were significant over the check AKT-8811. Hybrids of Dwarf 45 cm and Dwarf 90 cm were found to be good for yield

and heterosis. These lines will be useful in future hybrid breeding programmes.

A hybrid breeding technique based on the CGMS system was well developed and being utilized in pigeonpea. Based on this technique, three hybrids were released so far in Central India mainly in Madhya Pradesh, Maharashtra and Gujarat. Few CMS lines were developed from two cytoplasmic sources (A_2 and A_4) and regularly utilized in different breeding programmes with limited range of heterosis. Similarly, CMS line AK-120-1A based on A_2 cytoplasm was used mostly in different hybrid combinations with limited heterosis. Through present investigation, different height variant genes were combined with CMS to enhance the variation in genetic stalk of cytoplasmic male sterility. Generally, CMS lines are tall and similar to the restorer lines for height. These variable height CMS line will be helpful in maintaining the purity of the CMS lines and also to obtain heterotic combinations.

Male sterile line AK-120-1A exhibited white translucent anthers which are characterized by the absence of pollen grains. During the backcrossing programme translucent anthers were easily transferred to the four dwarf CMS lines. This trait was closely associated with the complete male sterility against shriveled anthers. The recessive nature of dwarfing genes of four recurrent parents was observed in F_1 and BC_1F_1 generations. Also dominant fertility restorer gene was absent in recurrent parents hence lines were directly used as a maintainer line for four new CMS lines.

Complete sterility was observed in F_1 generation which was declined in the next generations. This might be due to interaction between the two genomes and various other cytoplasmic- interactions which lead to variable expression of male sterility. Such

negative interaction effects reduce gradually by each passing generation of selection and backcrossing (Saxena and Kumar, 2003). In BC_5F_1 generation, 100% sterility was recovered in all the lines. The cytoplasmic male sterility based on A_2 cytoplasm was blended completely with four dwarf genotypes through backcross. In the characterization, lines were distinct from each other majorly for height and for many other traits. Characterization of new lines will help in the predicting the performance of progenies in the different breeding programme.

Fertility restoration study showed the presence of fertility restoring genes in the restorer lines for dwarf CMS lines. Seventeen hybrids were found completely fertile while the remaining hybrids were partially fertile. Nadarajan *et al.*, (2008) and Saxena *et al.*, (2010) reported variable restoration patterns among a common set of male parents within a single cytoplasmic source of pigeonpea. In pigeonpea the fertility restoring genes are sporophytic in nature (Dalvi *et al.*, 2008). Some modifier genes were present in the nucleus that may influence the process of penetrance and expressivity of the fertility restoring genes (Hossain *et al.*, 2010). Therefore, to obtain high pollen bearing plants with stable fertility restoration, selection for both the dominant fertility restoring genes is essential (Saxena *et al.*, 2011).

Newly developed CMS lines were tested in different hybrid combinations to know the utility in heterosis breeding. Diverse parents were given heterotic combinations over both the parents reveals the potential of the dwarf parents in heterosis breeding. Results showed that the hybrids Dwarf 45 cm x AKPR-344 (63.26 g) and Dwarf 45 cm x AKPR-325 (62.21 g) showed higher per se performance over both the parents and significant economic heterosis for grain yield per plant

over check PKV-TARA. These new dwarf CMS lines performed well in different hybrid combinations and also gave a good response to fertility restoration with different restorer lines. This showed that the dwarf CMS lines are useful for future breeding programme.

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