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Exploitation of the Leaf Variants in CGMS System Derived from A_2 Cytoplasm in Pigeonpea [*Cajanus cajan* (L.) Millsp.]

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A B S T R A C T

In the present study, cytoplasmic male sterility derived from A_2 cytoplasm of pigeonpea was combined with four leaf type agronomic backgrounds by using backcrossing programme These four leaf shapes were recessive in the expression against lanceolatus leaf shape. These naked eye polymorphic (NEP) markers will be useful for maintaining purity of hybrid seeds if coupled with cytoplasmic male sterility hence present investigation was conducted. In flower structure, keel petals were found open in Obcordifoliate leaf parent similar trait was transferred into respective CMS line, this trait will help in enhancing hybrid seed production and reduces its cost. Different hybrid combinations were made using new leaf type CMS and hybrids made by using obcordifoliate leaf showed highest seed yield over standard checks. Restorer lines used in the present study effectively restored the fertility new CMS lines. Performance of leaf type CMS in different hybrid combinations showed wide scope for utilizing these naked eye polymorphic (NEP) markers in future pigeonpea hybrid breeding program that will certainly help to maintain the genetic purity of CMS lines and quality of hybrid seed.

Introduction

Pigeonpea is a drought tolerant crop and one of the important grain legumes for semi-arid tropics, also called as poor man's meat due to its high protein content. Pigeonpea is a self-pollinated crop, but cross-pollination occurs up to 70%, mainly due to insect activity (Saxena *et al.*, 1990). In pigeonpea more than 100 varieties were release so far (Saxena *et al.*, 2006) with that area and production increased in last three decades, but productivity is hovering around 700±50 kg/ha (Saxena and Nadarajan, 2010). For breaking the yield plateau, hybrid breeding will be an important alternative.

First GMS system was developed by Reddy *et al.*, 1978. Six hybrids derived from the GMS system were released so far with 30-50% of yield advantage over the high yielding checks. Even though, hybrids were not commercialized due to the problem of roughing, that mainly increases cost of seed production and impurities in hybrids seeds. Another source of male sterility factor located in the cytoplasm was identified and eight different sources [designated as A_1, A_2, A_3, A_4, A_5, A_6, A_7 (Saxena *et al.*, 2010) and A_8 (Saxena *et al.*, 2013a) so far were utilized to develop CMS lines in pigeonpea. From these sources only two, *C. scarabaeoides* (A_2) (Tikka *et al.,* 1997) and *C. Cajanifoliuos* (A_4) (Saxena *et al.,* 2005) were successfully used to develop stable CGMS systems in pigeonpea. Through the CGMS system, three hybrids [GTH-1 (Acharya *et al.,* 2008), ICPH 2671 (Saxena *et al.,* 2013b) and ICPH 2740(Saxena *et al.,* 2013c)] were released in India. Even though, very less improvement was occurred in the productivity of pigeonpea. In most of the breeding programme common CMS lines were used. There is need to utilize diverse set of CMS lines and to enhance the genetic backgrounds of CMS line through backcrossing. In the present work, CMS line AK-120-1A was used as CMS source to diversify into four different leaf types. In addition, different hybrid combinations were made to identify fertility restorer gene and heterotic combiner parents from new leaf type CMS lines. Recently, Saxena *et al.,* (2011) incorporated obcordifoliate leaf shape into A_4 cytoplasm based CMS lines for use as naked eye polymorphic markers. The success of hybrid technology largely dependent on the provision of quality F_1 seeds to the farmers. It involves the application of principles of seed production to assure true F_1 from the seed production plots. Leaf shape variants will serve as naked eye polymorphic trait and will ensure absolute purity of hybrid seed as it assists in ease of rouging of off-types from A-lines. Self-plants and out crossed plants roughed out within 6-8 weeks after sowing from hybrids and CMS lines respectively (Patil *et al.,* 1998).present investigation was carried out to find the role of leaf types in future hybrid breeding programme.

Materials and Methods

Location of experiment

The present research work was carried out at research field of Pulses Research Station, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra State (India). It is Deccan plateau with black clayey soils and average annual rainfall of 825.3 mm. It is situated at Latitude 20º 42′ 10.59″N, Longitude 76º 59′ 57.97″E and Altitude 285 m. Annual temperatures range from a high of 48 °C (118 °F) to a low of 10 °C (50 °F). Akola is located on the Tropic of Cancer and becomes very hot during the summer, particularly in May.

Diversification programme

Germplasm lines with oval, sesamum, obcordifoliate and small leaf shapes (Fig. 1), were crossed with CMS line AK-120-1A (A_2 cytoplasm) in rainy season 2005. These leaf types are the germplasm lines collection at Pulses Research Station, Akola (India). During rainy season 2006, each individual plant of four F_1 generations was observed for the male sterility. Male sterile plants with white translucent anthers (Fig. 3d) were identified and used in backcrossing with recurrent parents in each cross. In BC_1F_1, plants were segregated into two categories for lanceolatus leaf and other leaf types, from that only male sterile plants with four leaf types were selected for backcrossing and remaining plants were discarded. Plant to plant sib mating was did during backcrossing. Male sterile plants with four leaf types were selected and backcrossed with recurrent parent up to BC_6F_1 generation in respective crosses. Schematics of backcrossing method used for diversification of oval leaf types into CMS background is presented in figure 2. The similar breeding scheme was utilized for another three leaf types.

For identification of sterile plants, pollens were observed for sterility/fertility by using 1% Potassium Iodide Iodine (KII) stain. In this, anthers were collected randomly from 3-5 flowers of each plant at 50% flowering and examined under simple microscope. The complete stained (deep blue colored) pollen grains were considered as fertile (Fig. 3b) however unstained pollen grains (Fig. 3a) and anthers with devoid of pollen grains (Fig. 3c) were considered as sterile. Another trait anther color also recorded for each plant 3-4 flowers, white translucent color of anther shows sterile with devoid of pollen grains and yellow color anthers is fertile.

Characterization of CMS lines

After getting 100% male sterility and uniform morphology these new CMS lines along with maintainer lines were observed for different characters such as sterility, anther color, seed color, plant growth habitat, flower color, standard petal streaks, pod streaks, days to 50% flowering, plant height and number of primary branches. Evaluation for yield and yield contributing traits was done during the rainy season, 2011 in replicated trial. Yield related traits such as number of days to maturity, pods per plant, grains per pod, 100 seed weight (g) and grain yield per plant (g) were observed from respective maintainer lines (B-line).

Evaluation of hybrids for fertility restoration and heterosis

Twelve restorer lines viz., 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 were used to generate 48 hybrid combinations in newly CMS lines for study performance in fertility restoration and heterosis. Crossing was done during Rainy season 2010 and there was sufficient pod setting.

These 48 hybrid combinations along with 12 restorer lines, 4 maintainer lines, and two high yielding local varieties as checks PKV-TARA and AKT-8811 were evaluated in randomized complete block design (RCBD) in three replications during rainy season, 2011. Row to row and plant to plant spacing was 60 cm and 20 cm respectively. Other packages of practices were adopted as per recommendations and requirements. Under the fertility restoration study, hybrids were observed for anther color, pollen fertility, and anther dehiscence. Fertility of pollen grains from every F_1 plants of respective hybrids was checked by using 1% KII stain. At the same time, observations on yield and yield contributing traits were recorded.

Results and Discussion

Diversification of CMS

In the present study, CMS source was used from AK-120-1A line, which was derived from A_2 cytoplasm. This line exhibited white translucent anthers showed complete male sterility without pollen grains, this trait was easily transmitted to the progeny plants of four F_1 crosses. The CMS line also exhibited lanceolatus leaf shape. Similar to the CMS parent all the plants in four F_1 populations exhibited normal lanceolatus leaf shape, showed the dominance expression of lanceolatus leaf over four leaf types

In four F_1 generations (2006-07), 34 plants (72%) of oval leaf cross, 14 plants (57%) of sesamum leaf cross, 29 plants (100%) of obcordifoliate leaf cross and 22 plants (100%) of Small leaf cross were found sterile, all the sterile plants exhibited translucent anthers without (Table 1). In BC_1F_1 generation, frequencies of progeny plants with lanceolatus leaf and four leaf shapes were observed in 1:1 ratio in respective crosses (Fig. 2). These observed frequencies were tested in a chi-square analysis for goodness of fit in 1:1 ratio and it was observed that these observed frequencies were fitted in the expected ratio (Table 2). Lanceolatus leaf shape and other four leaf types were segregated in 1:1 ratio, which is similar to the Mendelian test cross segregation. Results of male sterility observed during the present investigation are presented below.

In BC_1F_1 generation of oval leaf CMS, 16 oval leaf plants were found sterile among 26 plants, these oval leaf sterile plants were used in further back crossing with recurrent parent while other plants were discarded. In the next generation (BC_2F_1), 96.43% male sterile plants were observed with oval leaf. Few plants with less than 20% fertile pollen grains were found and were discarded. In the subsequent generations (BC_3F_1, BC_4F_1, BC_5F_1 and BC_6F_1), 100% male sterile plants with the plant type of oval leaf parent was recovered. Initially 72% male sterility was observed in F_1 generation, however at the end of BC_6F_1, 100% male sterility with oval leaf plant type was recovered through sib mating in each backcross generation.

In sesamum leaf backcrossing, 13 plants with sesamum leaf were found male sterile in BC_1F_1 generations. After using these sterile plants in BC_2F_1 generation, 99.3% male sterile plants were observed and 100 percent plants exhibited sesamum leaf shape. Fertile plants were discarded. In subsequent generations of backcrossing (BC_3F_1, BC_4F_1, BC_5F_1, and BC_6F_1), 100% plants were found male sterile with sesamum leaf type. Similarly, for obcordifoliate leaf CMS, 100% sterility was observed in BC_1F_1 and among them 16 plants were exhibited obcordifoliate leaf. In later generations of backcrossing (BC_2F_1, BC_3F_1, BC_4F_1, BC_5F_1, and BC_6F_1 generations) male sterile plants with obcordifoliate leaf was recovered. While backcrossing small leaf shape to cytoplasmic male sterile background, 14 plants with small leaf were found male sterile in CB1F1 generation and all later generations of backcrossing exhibited 100 % male sterile plants with small leaf shape. In the present study, four leaf types were combined with cytoplasmic male sterility source from A2 cytoplasm through repeated backcrossing and four new CMS lines with naked eye polymorphic leaf shape markers were developed to use in future hybrid breeding.

Descriptions of morphological traits

Characterization of newly developed breeding lines is important that will help breeders to select the desirable parents and to predict the performance of the progenies in the different breeding programmes. In the present study, new CMS lines observed for various morphological characters and description is given in the table 3. Leaf types itself is the naked eye polymorphic marker were tagged with male sterility in respective lines. There is difference in the anther color of four CMS line and respective B-lines. CMS lines exhibited white translucent anthers, whereas maintainers revealed yellow anthers. Pollen grains were absent in the translucent anther (Fig. 2c) and present in ample amount in yellow anther. In all the lines pigmentations on stem were absent similarly pigmented streaks on the standard petal of flower was absent or faint in color in the four CMS lines and maintainer lines. In pigeonpea, keel petals of the flowers are mostly closed in the present cultivars, such closed structure also observed in oval, sesamum and small leaf that enhances the self-pollination. However, open keel structure was observed in the recurrent parent of Obcordifoliate leaf and same trait was transmitted to Obcordifoliate leaf CMS line. Open keel of flower may enhance the cross pollination for maximum seed setting under open pollination. Purple pigments on the pods were present in sesamum and obcordifoliate leaf lines, while such pigments on pods were absent in oval leaf and small leaf lines. White colored and bold seeds (11.41±0.11g) were observed in Oval leaf line. Seed size of other three lines was ranges from 8 to 9 g. Maturity period of new lines is medium in duration (140-150 days) (Table 2). Number of pods and seed yield per plant is maximum in Obcordifoliate leaf (110.1±14.5 and 30.89±3.2 g resp.) followed by Small leaf (102.11±13.1 and 30.51±2.3 g resp.), Oval leaf (62.87±12.4 and 27.99 ±4.2g resp.) and Sesamum leaf (58.57±9.8 and 15.65±2.1 g resp.).

Fertility restoration in leaf variant CMS lines

Utilization of any cytoplasmic male sterile line in hybrid breeding programme depends on availability of male fertility restorer gene/s in the population. Generally, fertility restorer genes are available and need to search from the population or from germplasm (Saxena and Kumar, 2003). In the present study, 12 restorer lines were used to identify fertility restoring gene for new leaf type CMS lines. New CMS lines revealed good response to fertility restoration with good pollen fertility. Ample quality of pollen grains and considerable pod setting in the 48 hybrids, showed effective fertility restoration in leaf type CMS lines. Among the 48 hybrids, 19 were found 100% male fertile and remaining 29 hybrids were showed partially fertility (Table 4). None of the hybrid was found completely sterile. Among the leaf types, obcordifoliate leaf CMS found good in fertility restoration and exhibited maximum number (8 hybrids) of complete (100%) fertile hybrids with high pollen fertility score.

Highest pollen fertility score 90.01±3.42%) was observed in hybrid Small leaf CMS x AKRP-249. Complete fertile hybrids showed range of 81.01±1.68% to 90.01±3.42% for pollen fertility score. Two restore lines (AKPR-249 and AKPR-319) were found good for fertility restoration in all the four leaf type CMS lines.

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

Year	Generations	Sterility of Recurrent Parents (%)			
		Oval leaf	Sesamum leaf	Obcordifoliate leaf	Small leaf
2006-07	F_1	72.41 (34 plants)	57.57 (14 plants)	100 (29 plants)	100 (22 plants)
2007-08	BC_1F_1	89.65 (26 plants)	56.32 (13 plants)	97.14 (34 plants)	100 (31 plants)
2008-09	$BC_2 F_1$	96.43 (27 plants)	96.77 (30 plants)	100 (23 plants)	100 (36 plants)
2009-10	BC_3F_1	100 (122 plants)	100 (138 plants)	100 (118 plants)	100 (141 plants)
2010-11	BC_4F_1	100 (152 plants)	100 (168 plants)	100 (129 plants)	100 (184 plants)
2011-12	BC_5F_1	100 (166 plants)	100 (185 plants)	100 (144 plants)	100 (214 plants)
2012-13	BC_6F_1	100 (189 plants)	100 (206 plants)	100 (164 plants)	100 (225 plants)

Note: (In bracket) – No. of sterile plants observed

Table.2 Chi- square test for segregation in the BC_1F_1 generation

Cross	Total plants	Observed Frequency		Expected Frequency (1:1)		DF	$(O-E)^2$		χ2 Value	P Value	
		Lanceolatus leaf	Leaf Variants	Lanceolatus leaf	Leaf Variants		Lanceolatus Leaf	Leaf Variants			
Lanceolatus leaf (AK-120-1A) x Oval leaf	29	13	16	14.5	14.5	1	0.155	0.155	0.310	0.46	NS
Lanceolatus leaf (AK-120-1A) x Sesamum leaf	23	10	13	11.5	11.5	1	0.196	0.196	0.392	0.46	NS
Lanceolatus leaf (AK-120-1A) x Obcordifoliate leaf	35	19	16	17.5	17.5	1	0.129	0.129	0.258	0.46	NS
Lanceolatus leaf (AK-120-1A) x Small leaf	31	17	14	15.5	15.5	1	0.145	0.145	0.290	0.46	NS

Table.3 Description characters of different new A- lines and their respective maintainers recorded at Akola

Characters	Oval leaf-A	Oval leaf-B	Sesamum leaf-A	Sesamum leaf-B	Obcordifoliate leaf-A	Obcordifoliate leaf-B	Small leaf-A	Small leaf-B
Sterility %	100	0	100	0	100	0	100	0
Anther color	White Translucent	Yellow	White Translucent	Yellow	White Translucent	Yellow	White Translucent	Yellow
Flower Color	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
Petal streaks	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Keel Structure	Closed	Closed	Closed	Closed	Open	Open	Closed	Closed
Pod Streak	Absent	Absent	Present	Present	Present	Present	Absent	Absent
Seed color	White	White	Brown	Brown	Brown	Brown	Brown	Brown
Growth Habit	IDT	IDT	IDT	IDT	IDT	IDT	IDT	IDT
Branching pattern	Semi spreading	Semi spreading	Erect	Erect	Semi spreading	Semi spreading	Semi spreading	Semi spreading
Days to 50% flowering	104 ±0.7	106 ±0.3	116 ±0.7	118 ±0.3	112 ±0.1.3	112±0.7	120 ±0.3	122 ±1.3
Days to Maturity	-	151 ±1	-	151 ±0.6	-	159 ±0.7	-	151 ±0.7
Height (cm)	129.1 ±3.4	128.2 ±2.5	109.5 ±2.1	108.9 ±4.1	112.6 ±3.8	113.6 ±5.1	125.6 ±3.4	127.1±4.8
No. of Primary	10.13 ±3.2	9.97 ±2.8	8.25 ±4.2	9.12 ±2.5	7.33 ±3.2	8.23 ±2.9	10.88 ±4.2	12.24±1.9
No. of pods/plant	-	62.87±12.4	-	58.57 ±9.8	-	110.1±14.5	-	102.11±13.1
Seed per Pod	-	3.64 ±0.02	-	3.1±0.03	-	3.34±0.01	-	3.41±0.01
100 Seed Weight (g)	-	11.41±0.11	-	8.74±0.13	-	8.46±0.05	-	8.55±0.04
Yield per Plant (g)	-	27.99 ±4.2	-	15.65±2.1	-	30.89±3.2	-	30.51±2.3

Note: IDT- Indeterminate growth habit, %- percent.

Table.4 Fertility restoration and pollen fertility score of different hybrids

Restorers	Oval leaf		Sesamum Leaf		Obcordifoliate leaf		Small Leaf	
	F.R.%	P.F.S.%	F.R.%	P.F.S.%	F.R.%	P.F.S.%	F.R.%	P.F.S.%
AKPR-249	91.67	76.96±3.67	100	85.81±2.25	100	84.63±1.66	100	90.01±3.42
AKPR-178 (E)	90	69.75±3.33	81.25	66.71±2.36	79.49	69.82±1.75	82.26	63.26±4.22
AKPR-12	79.07	67.35±2.29	84.91	67.64±0.85	79.17	67.06±5.78	94.23	72±3.61
AKPR-178 (M)	88.37	82.14±7.90	92.5	80.24±0.48	100	88.16±2.11	100	84.51±1.54
AKPR-344	87.1	73.15±3.57	95.74	72.06±1.95	93.33	78.17±2.72	83.72	65.49±1.39
AKPR-8	91.84	76.43±3.48	100	85.96±3.62	89.47	77.13±4.54	69.57	55.16±1.87
AKPR-210	94.87	84.57±2.43	100	86.05±2.34	100	85.06±1.45	95.35	67.2±2.58
AKPR-325	100	82.85±2.52	94.29	75.88±2.08	100	82.77±1.85	82.61	69.51±2.72
AKPR-364	100	85.34±2.67	89.47	70.81±6.42	100	88.21±3.35	75.51	62.44±1.12
AKPR-319	95.92	83.61±1.54	100	84.32±3.11	100	81.01±1.68	100	83.85±0.64
AKPR-359	91.18	80.55±3.16	87.5	76.28±1.09	100	84.47±4.24	100	84.98±0.98
AKPR-292	94.44	79.83±2.45	100	82.22±1.33	100	82.47±2.95	94.59	82±4.16

Note: F.R.%- Percent fertility restoration, P.F.S.%- Percent pollen fertility score.

Table.5 P*er se* performance of top performing hybrids for different yield and yield contributing trait with economic heterosis

Hybrids	Days to Maturity	Seeds per Pod	100 Seed Weight (g)	No. Of pods plant^{-1}	Seed yield per plant	Economic Heterosis	
						H3a	H3b
Obcordifoliate leaf A X AKPR-344	142	3.55	7.82	271.43	64.47	21.70 **	68.90**
Obcordifoliate leaf A X AKPR-178 (M)	145	3.39	8.65	240.33	64.2	21.19 **	68.19**
Obcordifoliate leaf A X AKPR-210	138	3.34	12.21	139.6	56.38	6.43 *	47.71**
Oval leaf A X AKPR-359	144	3.35	10.13	176.28	54.07	2.06	41.66**
Sesamum leaf A X AKPR-364	138	3.46	9.24	168.43	53.6	1.18	40.42**
Obcordifoliate leaf A X AKPR-249	131	3.38	7.28	217.73	52.56	-0.78	37.70**
Obcordifoliate leaf A X AKPR-12	142	3.29	8.46	182.97	50.95	-3.83	33.48**
Oval leaf A X AKPR-364	142	3.56	9.42	170.51	50.69	-4.32	32.80**
Oval leaf A X AKPR-292	154	3.51	9.41	149.38	48.21	-9.00 **	26.30**
Small leaf A X AKPR-292	144	3.49	10.16	129.34	45.26	-14.56 **	18.57**
Sesamum leaf A X AKPR-178 (M)	144	3.29	8.36	165.43	45.22	-14.64 **	18.47**
PKV-Tara (Check)	162	3.6	10.47	155.87	52.98		
AKT-8811 (Check)	140	3.75	11.22	144.78	38.17		
SEm	±3.67	±0.01	±1.2	±13.94	±6.27		

Note: * and **- Significant at 5 and 1 % level of significance, H3a- Economic heterosis over check PKV TARA, H3b- Economic heterosis over check AKT-8811.

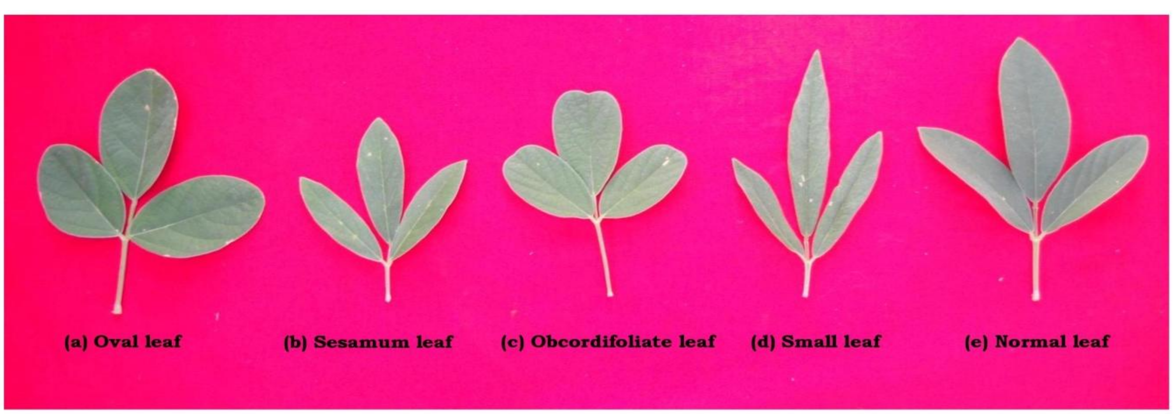


Fig 1: Different types of leaf shapes used in diversification (a) Oval leaf (b) Small leaf (c) Obcordifoliate leaf (d) Sesamum leaf (e) Normal leaf (Lanceolatus leaf)

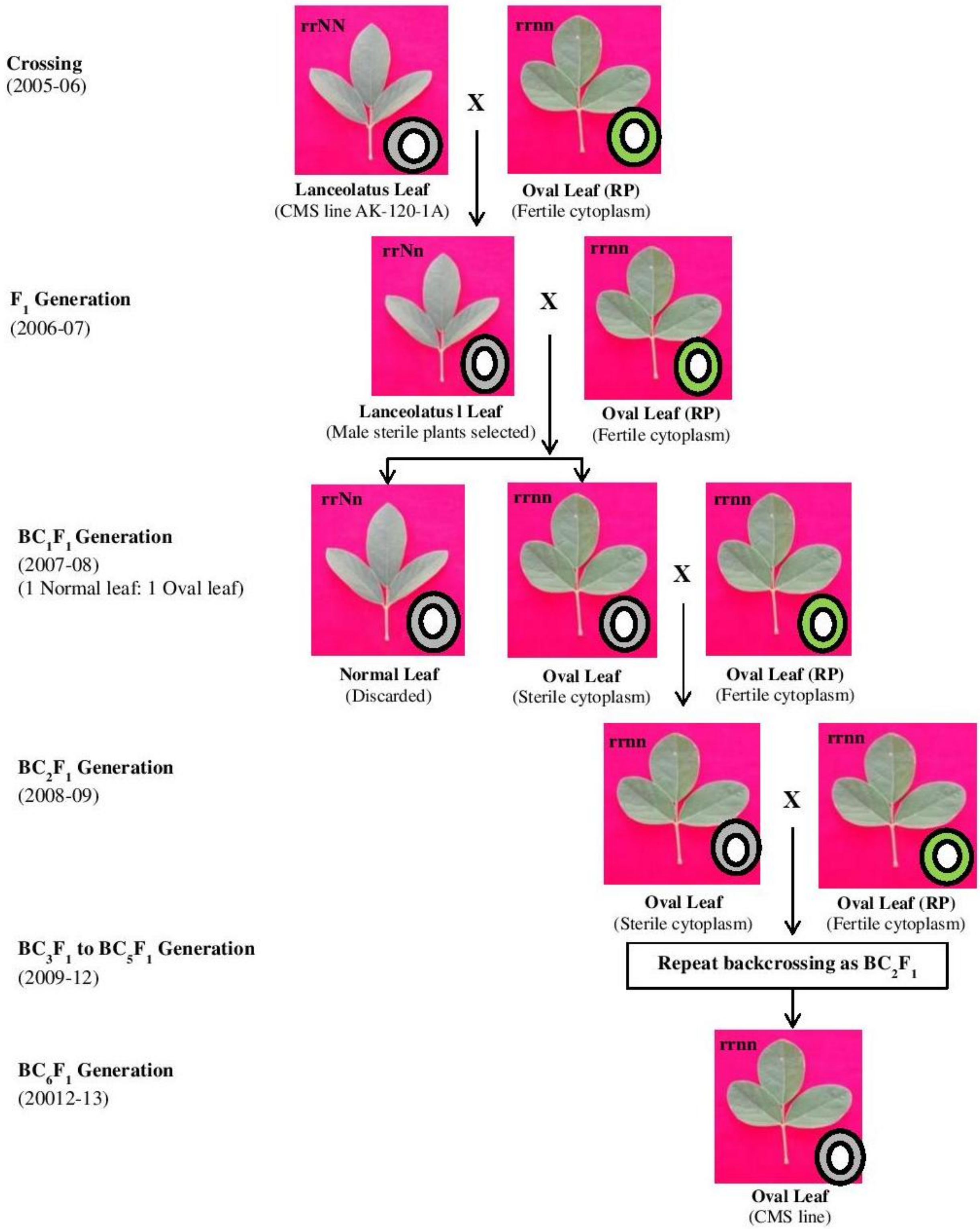
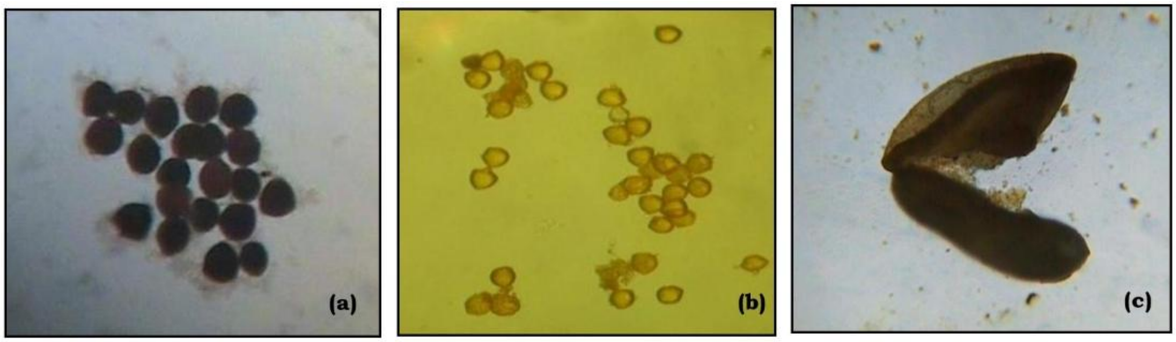


Fig 2. Schematic of backcrossing method used for diversification of CMS into Oval leaf parent (r - Recessive fertility gene, N- dominant gene govern lanceolatus leaf shape, n- recessive gene govern oval leaf shape)



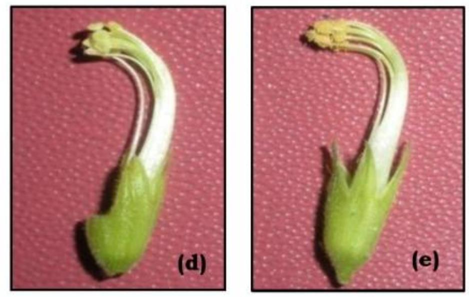


Fig. 3: Differences of pollen grain in KII staining
(a) Fertile Pollen Grain (Stained),
(b) Sterile Pollen Grain (Unstained),
(c) White Translucent Anther (Devoid/ absence of pollen grains)
(d) White translucent anther
(e) Yellow color anther

Hybrid performance

Performance of hybrids derived from new CMS lines will decide the prospects of the CMS line in the hybrid breeding. In the present study, 48 hybrids developed on new CMS lines were evaluated and only promising hybrids based on *per se* performance is presented in the table 5. A significant amount of heterosis was observed in the hybrids developed on new CMS lines showed the prospects of leaf type CMS lines for future hybrid breeding. For grain yield per plant, twenty-seven and twenty-one hybrids exhibited significant average heterosis and heterobeltiosis respectively. Among the forty-eight hybrids, three were superior to high yielding check PKV-TARA and twelve hybrids were superior over another check AKT-8811 for seed yield per plant. Among the significant hybrids, Obcordifoliate leaf x AKPR-344 exhibited highest economic heterosis (21.70%) over check PKV-TARA.

It is often said that pigeonpea has reached its performance plateau (Saxena, 2008). There is a huge gap between the potential yield and actual yield of pigeonpea. Even after releasing six GMS based and three CGMS based hybrids in India, productivity is hovering around 0.7-0.8 t ha^{-1}. Stable CGMS technology has not made any big impact in the production and productivity of pigeonpea till now, but the prospect of CGMS hybrid breeding was found much more than GMS and traditional breeding techniques. In the present investigation, different leaf types were combined with cytoplasmic male sterility to broaden the genetic base for hybrid breeding. In the first generation (F_1) these four leaf types were found recessive in expression against lanceolatus leaf. Recessive gene expression for Obcordifoliate leaf shape was observed by Cruz *et al.*, (1971), Deokar *et al.*, (1972), Chopde *et al.*, (1979) and Saxena *et al.*, (2011). Most of the F_1 plants were found sterile as CMS line; it indicated that the factor for male fertility is located in cytoplasm of four recurrent parents. Hence, four recurrent parents were directly used as maintainer line for respective leaf type CMS line.

These leaf types are recessive traits were combined with female lines for use as naked eye marker for maintaining the purity of CMS lines. Earlier Saxena *et al.*, (2011) combined

Obcordifoliate leaf with a CMS line of A_4 cytoplasm and called it as a naked eye polymorphic marker. In BC_1F_1 generation, four leaf types were segregation in 1:1 ratio with lanceolatus leaf, which revealed recessive expression of leaf types. Similarly Chopde *et al.*, (1979) reported 1:1 ratio for segregation of Obcordifoliate leaf and lanceolatus leaf. Few generations of backcrossing were taken to recover recurrent parent genotype with male sterility due to the sib mating between sterile plants and recurrent parent. At end of the backcrossing programme successfully developed four leaf type CMS lines from A2 cytoplasm. Saxena and Kumar (2003) stated that the experimental hybrids derived from the A_1 and A_2cytoplasms, were shy bearers and produces less pollen, but in the present experiment, hybrids derived from leaf type CMS lines of A_2 cytoplasm produced good quantity of pollen grains with high pollen fertility.

Open flower structure was observed in the Obcordifoliate leaf CMS and maintainer that will enhance the hybrid seed production in the isolation. Restorer lines used in the present study exhibited fertility restoring gene for leaf type CMS lines. Nadarajan *et al.,* (2008) and Saxena and Kumar (2003) identified restorer gene from available germplasm lines for different CMS lines. Among the forty-eight hybrids, nineteen hybrids were complete fertile (100%) while twenty-nine hybrids were partially fertile (69.57 - 95.92%). It was postulated that the partial fertility is mainly due to the partial expressivity of restorer genes in different CMS lines. Some modifier genes present in the nucleus and cytoplasm could be the reason for incomplete penetrance and expressivity of the fertility restoring genes (Hossain *et al.*, 2010). For getting effective fertility restoration Saxena *et al.*, (2011) postulated that the selection for both the dominant fertility restoring genes in one line is essential that will increase the restoration in the hybrids. Two restorers AKPR-249 and AKPR-319 were identified best for fertility restoration in four leaf type CMS lines.

Hybrids developed from leaf type CMS showed the over dominance expression over both the parents and gives significant standard heterosis over checks. It shows that there is a prospect for leaf CMS in the future hybrid breeding programme. Among the four leaf CMS lines, obcordifoliate leaf CMS was best for fertility restoration and heterosis. There is a possibility of having a relationship of heterosis and fertility restoration with obcordifoliate leaf type, further confirmation is needed. Other three leaf types also found good for heterosis breeding. In the present investigation, new CGMS system derived from A_2 cytoplasm with different leaf types has produced for future breeding. These four leaf types CMS lines will add some benefit to future hybrid breeding of pigeonpea. Leaf types CMS lines are capable of producing heterotic hybrid combinations and will help in the increasing production and productivity.

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