Journal of Food Legumes 28(3): 215-219, 2015

Studies on stability and chacterization of early maturing CMS lines of pigeonpea (*Cajanus cajan* (L.) Millspaugh)

JITENDRA PANDAT, INDERJIT SINGH and SARVJEET SINGH

Punjab Agricultural University, Ludhiana, Punjab, India

E-mail: inderjitpau@rediffmail.com

(Received: 4 February 2015; Accepted: 26 November 2015)

ABSTRACT

The present investigation was conducted during *kharif* season of 2012 and 2013 in Department of Plant Breeding and Genetics, PAU, Ludhiana to study the stability and morphological characterization of 18 newly developed CMS lines in early background along with GT 288A (A_2 cytoplasm) and ICPA 2089 (A_4 cytoplasm) CMS lines. All the genotypes under study were found to be 100 per cent non pollen shedding i.e. completely male sterile at all dates during *kharif* season as well as during off-season. It indicated that expression of sterility of CMS lines was not affected by the environmental factors like temperature, relative humidity, sunshine hours and day length. Therefore, these CMS lines can be effectively exploited in hybrid development programme as these were stable for maintaining their sterility under varying environmental conditions. All the CMS lines were significantly early in flowering and maturity than GT 288A and ICP 2089A. Further, these were also significantly shorter in plant height than GT 288A and ICP 2089A. CMS lines also were identified promising for different traits viz. AL 117A for primary branches, AL 120A for secondary branches, AL 120A and AL103A for pods per plant, seeds per pod and grain yield per plant.

Key words: Characterization, Male sterility, Pigeonpea, Stability

Pigeonpea (*Cajanus cajan* (L.) Millspaugh) is the sixth most important food legume of the world and is grown on about 5 million hectares globally in about 50 countries of South Eastern Asia, Eastern Africa, Caribbean region, South and Central America. India has the largest acreage (4 million hectare) under pigeonpea and accounts for about 80 per cent of area of the world (Wanjari and Rathod 2012). It accounts for about 14 per cent of the area under pulses and 20 per cent of the pulses production in India. In India, the major pigeonpea growing states are Maharashtra, Karnataka, Andhra Pradesh, Uttar Pradesh, Gujarat and Madhya Pradesh which account for over 70 per cent of the total pigeonpea area and production. In Punjab it was grown on 3.1 thousand ha with production of 2.7 thousand tonnes (Anonymous 2013).

To promote pigeonpea cultivation and production, genetic improvement was emphasized by researchers for more than five decades and a number of cultivars have been developed through hybridization and selection of land races (Singh *et al.* 2005). However, the progress in the genetic improvement of yield potential has been limited and improved cultivars failed to enhance the productivity of this crop significantly. Therefore, an alternate breeding approach of hybrid technology was attempted in pigeonpea to enhance the yield. A substantial level of natural out-crossing in this crop provides opportunity for exploitation of hybrid vigour but it could not be utilised in commercial hybrid breeding due to non availability of male sterile source. In 1974, a genetic male sterility (GMS) source was identified and first GMS based hybrid ICPH 8 was released in 1991 in India (Saxena *et al.* 1992) followed by hybrids like PPH 4 in 1993 by PAU, Ludhiana (Verma and Sidhu 1995), CoH 1 and CoH 2 by TNAU, Coimbatore. Subsequently two more hybrids AKPH 4104 and AKPH 2022 were released by PDKV, Akola in 1997 and 1998, respectively (Wanjari *et al.* 1999). But due to high cost of hybrid seed production and problem in maintenance of male sterility (rouging of 50 per cent fertile plants from GMS parent), these hybrids could not be adopted at large scale.

To overcome the limitation of GMS based hybrids, the development of cytoplasmic genetic male sterility (CGMS) became imperative. Tikka *et al* (1997) and Saxena and Kumar (2003) identified cytoplasmic genetic male sterility (A_2) from wild species *Cajanus scarabaeoides*. So far, eight such systems from different wild species have been developed (Saxena *et al.* 2013). Of these, A_2 (*C. scarabaeoides*) and A_4 (*C. cajanifolius*) systems exhibited promise because of their stability under various agro-climatic zones and availability of good maintainers and fertility restorers. The strenuous efforts led to development of world's first CMS based pigeonpea hybrid GTH 1 utilizing GT 288A (A_2) by Gujarat Agricultural University, S.K. Nagar for Gujarat State, then ICRISAT developed ICPH 2671 a medium duration hybrid based on A_4 cytoplasm and was released for commercial cultivation in Madhya Pradesh in 2010 (Saxena *et al.* 2010).

In North India including Punjab state, pigeonpea crop has to compete with rice and cotton crop which offer high returns. Under Punjab conditions, there is need of early maturing CMS based hybrids with maturity period of 130-140 days to facilitate the timely sowing of wheat crop. The Punjab Agricultural University has developed 18 male sterile lines (A_2 cytoplasm) in early background and conversion programme of A_4 cytoplasm in early background is in progress. Before exploitation of these newly developed CMS lines there is need to study their stability and morphological characterization. So, the present study was planned o study the stability of newly developed CMS lines and their morphological characterization.

MATERIALS AND METHODS

The field experiment was conducted at Punjab Agricultural University, Ludhiana (Punjab) during *kharif* 2012 and 2013. Ludhiana, which represents the trans-Indo-Gangetic alluvial plains, is situated at the 30°56' N latitude and 75°52' E longitude and at an altitude of 247 m above mean sea level. The experiment comprised a set of 20 cytoplasmic male sterile (CMS) lines (18 new + GT 288A and ICP 2089A) along with their maintainer lines to check the stability of male sterility and morphological characterization. The experimental material was sown in Randomized Block Design with two replications in *kharif* 2012 and *kharif* 2013 in the experimental field area of Pulses Section, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. The treatments were accommodated in two-row plot of 2 m length with row-to-row spacing of 50 cm and plant to plant spacing of 25 cm. The recommended package of practices was followed to raise the crop. Individual plants from each male sterile line were observed at 15 days interval after the onset of flowering for pollen shedders and pollen viability with 2 per cent acetocarmine stain (Zhang *et al*, 2002) whereas, data for the morphological traits i.e. days to flowering, days to maturity were recorded on plot basis and the characters like primary branches, secondary branches, plant height, pods per plant, seeds per pod and 100-seed weight were recorded from five randomly taken plants. To test the significance of differences among genotypes (CMS lines), the mean data of different characters in each replication were statistically analyzed following randomized block design.

RESULTS AND DISCUSSION

In pigeonpea the development of stable cytoplasmic-genic male sterility (CGMS) system has opened up the possibility for production of commercial hybrids. The use of highly stable CMS system will reduce the cost of hybrid seed production by eliminating the task of emasculation.

Stability of male sterile lines

Pollen shedding (%): Pollen grains from mature anthers of different CMS lines were checked for pollen shedding at regular intervals from the onset of flowering. All the CMS lines under study were found to be 100 per cent non pollen shedding i.e. completely male sterile at all dates during *kharif* season as well as during off-season (Table 1a and Table 1b).

Pollen viability (%): Pollen grains from mature anthers of different CMS lines were studied under the microscope at regular intervals from the onset of flowering. All the CMS lines under study had complete pollen sterility (non viable pollen grains) and there was no variation amongst CMS lines for this trait. All the CMS lines under study had shown stable and complete stability for the expression of sterility.

Effect of environment on male sterility: Metrological data were recorded on various dates to see the effect of environment on pollen sterility of CMS lines during *kharif* season as well as off-season. Though the maximum

Table 1a. Pollen shedding (%) and Pollen viability (%) of various CMS lines at different time intervals in pigeonpea during *kharif* 2013

S. No.	CMS lines	Date of observation					
		21/08/2013		18/09/2013		20/10/2013	
		Pollen shedders (%)	Pollen viability (%)	Pollen shedders (%)	Pollen viability (%)	Pollen shedders (%)	Pollen viability (%)
1	AL 100A	0.0	0.0	0.0	0.0	0.0	0.0
2	AL 102A	0.0	0.0	0.0	0.0	0.0	0.0
3	AL 103A	0.0	0.0	0.0	0.0	0.0	0.0
4	AL 104A	0.0	0.0	0.0	0.0	0.0	0.0
5	AL 105A	0.0	0.0	0.0	0.0	0.0	0.0
6	AL 108A	0.0	0.0	0.0	0.0	0.0	0.0
7	AL 109A	0.0	0.0	0.0	0.0	0.0	0.0
8	AL 110A	0.0	0.0	0.0	0.0	0.0	0.0
9	AL 111A	0.0	0.0	0.0	0.0	0.0	0.0
10	AL 112A	0.0	0.0	0.0	0.0	0.0	0.0
11	AL 113A	0.0	0.0	0.0	0.0	0.0	0.0
12	AL 114A	0.0	0.0	0.0	0.0	0.0	0.0
13	AL 115A	0.0	0.0	0.0	0.0	0.0	0.0
14	AL 116A	0.0	0.0	0.0	0.0	0.0	0.0
15	AL 117A	0.0	0.0	0.0	0.0	0.0	0.0
16	AL 118A	0.0	0.0	0.0	0.0	0.0	0.0
17	AL 119A	0.0	0.0	0.0	0.0	0.0	0.0
18	AL 120A	0.0	0.0	0.0	0.0	0.0	0.0
19	GT 288A	0.0	0.0	0.0	0.0	0.0	0.0
20	ICP 2089A	0.0	0.0	0.0	0.0	0.0	0.0

Table 1b. Pollen shedding (%) and Pollen viability (%) of various CMS lines at different time intervals in pigeonpea during off-season (2014)

S. No.	CMS lines	Date of observation					
		15/02/2014		05/03/2014		20/03/2014	
		Pollen shedders (%)	Pollen viability (%)	Pollen shedders (%)	Pollen viability (%)	Pollen shedders (%)	Pollen viability (%)
1	AL 100A	0.0	0.0	0.0	0.0	0.0	0.0
2	AL 102A	0.0	0.0	0.0	0.0	0.0	0.0
3	AL 103A	0.0	0.0	0.0	0.0	0.0	0.0
4	AL 104A	0.0	0.0	0.0	0.0	0.0	0.0
5	AL 105A	0.0	0.0	0.0	0.0	0.0	0.0
6	AL 108A	0.0	0.0	0.0	0.0	0.0	0.0
7	AL 109A	0.0	0.0	0.0	0.0	0.0	0.0
8	AL 110A	0.0	0.0	0.0	0.0	0.0	0.0
9	AL 111A	0.0	0.0	0.0	0.0	0.0	0.0
10	AL 112A	0.0	0.0	0.0	0.0	0.0	0.0
11	AL 113A	0.0	0.0	0.0	0.0	0.0	0.0
12	AL 114A	0.0	0.0	0.0	0.0	0.0	0.0
13	AL 115A	0.0	0.0	0.0	0.0	0.0	0.0
14	AL 116A	0.0	0.0	0.0	0.0	0.0	0.0
15	AL 117A	0.0	0.0	0.0	0.0	0.0	0.0
16	AL 118A	0.0	0.0	0.0	0.0	0.0	0.0
17	AL 119A	0.0	0.0	0.0	0.0	0.0	0.0
18	AL 120A	0.0	0.0	0.0	0.0	0.0	0.0
19	GT 288A	0.0	0.0	0.0	0.0	0.0	0.0
20	ICP 2089A	0.0	0.0	0.0	0.0	0.0	0.0

temperature varied from 27.9 to 34.2°C and minimum temperature varied from 11.5 to 3.1°C during the flowering time, yet it had no effect on pollen shedding and pollen sterility. Relative humidityalso varied from 85.5 to 93.7 per cent during this period while sunshine hours and day length was recorded in the range of 4.2 to 10.3 hours. This lead to an important conclusion that expression of sterility of CMS lines under present study was not affected by the environmental factors like temperature, relative humidity, sunshine hours and day length and both A_2 and A_4 cytoplasms are quite stable against variation in environmental fluctuations. Saxena *et al* (2006) reported that A_4 cytoplasm to be stable for sterility in a multilocation study at Coimbatore, Hyderabad and Ludhiana. Gowda and Basha (2005) reported that two environmental male sterile lines PSMS 4-2 and PSMS 8-25 have shown almost 100 per cent pollen sterility during December when temperature were low and day length was short and when temperature and day length increased the pollen sterility decreases and vice-versa. Saxena (2009) reported that the process of conversion of male-sterile plants to male fertility started at the end of September and continued up to middle of November. Also, it was observed that there was a genetic variation for this trait among and within progeny. Such converted male-fertile plants reverted back to male-sterility in the month of February. These observations suggested that shortening of day lengths and reduction in temperatures induced male fertility, while high temperatures and longer days maintained male sterility. The newly developed CMS lines under investigation were in general early maturing and highly stable for expression of male sterility in varied environmental conditions and therefore, can be used in commercial hybrid breeding programme.

Morphological characterization of male sterile lines:

The pooled analysis of variance also revealed highly significant differences among genotypes for all the characters. The mean sum of squares due to environment were also significant for all the traits, except secondary branches per plant, pods per plant and seed per pod, indicating diverse nature of environments. (Table 2).The genotype x environment interaction was significant for all the characters, except for days to maturity, plant height and grain yield per plant, indicating differential response of the genotype to different environments The *per se* performance of different CMS lines for various characters studied is presented in Table 3.

Days to 50 per cent flowering: Flowering is very important character as synchronization is required among A (male sterile), B (maintainer) and R (restorer) lines for the successful production of hybrid seed and for maintenance of A lines. Days to 50 per cent flowering ranged from 88 to 119 days. All the CMS lines were significantly earlier in flowering than GT 288A (119 days) and ICP 2089A (114 days). Among the CMS lines, AL 112A (88 days) was earliest to have 50 per cent flowering. Six CMS lines namely, AL 102A (90 days), AL 104A (89 days), AL 108A (91 days), AL 110A (89 days), AL 111A (89 days) and AL 116A (91 days) were statistically at par with AL 112A (88 days). It was observed that all the maintainer lines (B lines) were earlier in flowering than their respective CMS lines.

Days to maturity: Days to maturity ranged from 136 to 162 days. All the CMS lines were significantly earlier in maturity than GT 288A (162 days) and ICP 2089A (155 days). Among the CMS lines, AL 112A (136 days) was earliest to attain maturity. The CMS lines AL 102A, AL 110A and AL

Table 2. Analysis of variance (mean squares) for various characters in different CMS lines of pigeonpea (Pooled over years)

Source of variation	d.f.	Mean Squares				
		Days to 50 % flowering	Days to maturity	Plant height (cm)	Primary branches per plant	Secondary branches per plant
Replications (Within Env)	2	2.31	9.28*	83.10	3.02	5.01
Environments	1	23.12*	33.75**	277.50*	14.45*	13.61
Treatments	19	256.45**	152.32**	1077.97**	24.72**	74.98**
Interaction	19	9.13*	4.72	113.10	8.94**	14.92*
Error	38	4.81	2.77	66.63	3.20	7.27
Total	79	295.82	202.84	1618.30	54.33	115.79

Source of Variation	d.f.	Mean Squares			
		Pods per plant	Seeds per pod	100-seed weight (g)	Grain yield per plant (g)
Replications (Within Env)	1	323.43	0.01	0.02	19.43
Environments	1	288.50	0.01	0.14*	287.56*
Treatments	19	8223.71**	0.18**	0.76**	552.12**
Interaction	19	992.84**	0.03**	0.07**	36.45
Error	38	266.92	0.01	0.03	45.31
Total	79	10095.40	0.24	1.02	940.87

* Significant at 5%, ** Significant at 1%

Table 3. Mean performances of different morpho-physiological traits of CMS lines during *kharif* 2012 and 2013

CMS Line	Days to 50 % flowering	Days to Maturity	Plant height (cm)	Primary branches per plant	Secondary branches per plant	Pods per plant	Seeds per pod	100-seed weight (g)	Grain yield per plant (g)
AL 100A	97	145	246	12	20	204	3.7	7.6	52.2
AL 102A	90	138	228	15	17	164	3.7	8.0	47.5
AL 103A	94	143	240	17	26	256	3.9	8.1	61.7
AL 104A	89	140	226	11	16	149	3.5	7.0	32.3
AL 105A	94	144	239	14	16	128	3.5	8.1	33.0
AL 108A	91	140	213	12	18	220	3.4	7.3	42.5
AL 109A	93	142	231	16	17	137	3.4	7.7	31.1
AL 110A	89	138	205	13	18	138	3.4	6.9	30.3
AL 111A	89	138	221	16	14	214	3.5	7.6	52.0
AL 112A	88	136	237	13	19	169	3.7	8.1	49.1
AL 113A	95	143	223	16	18	135	3.4	7.6	33.3
AL 114A	97	146	217	12	14	141	3.8	8.0	43.2
AL 115A	94	142	243	15	23	201	3.3	8.1	50.4
AL 116A	91	140	245	15	14	156	3.4	7.3	38.1
AL 117A	93	141	230	22	23	188	3.8	8.1	55.2
AL 118A	102	149	200	13	12	88	3.3	7.3	21.4
AL 119A	92	141	219	13	17	128	3.4	7.6	31.0
AL 120A	93	142	228	16	28	260	3.9	8.3	61.9
GT 288A	119	162	265	17	25	204	3.9	8.5	58.3
ICP 2089A	114	155	258	16	20	144	3.8	8.1	40.1
Mean	96	143	231	15	18	171	3.6	7.8	43.2
Range	88-119	136-162	200-265	11-22	12-28	88-260	3.3-3.9	6.9-8.5	21.4-61.9
C.V.	2.73	1.16	3.54	12.23	14.41	9.55	2.86	2.34	15.57
C.D. (p=0.05)	3.14	2.38	11.69	2.56	3.86	23.39	0.15	0.26	9.63

111A (138 days) were statistically at par with AL 112A (136 days). It was observed that all the maintainer lines (B lines) were earlier to attain maturity than their respective CMS lines.

Plant height (cm): Plant height ranged from 200 to 265 cm. All the CMS lines were significantly shorter in plant height than GT 288A (265 cm) and ICP 2089A (258 cm). CMS line, AL 118A (200 cm) was shortest in plant height followed by AL 110A (205 cm) and AL 108A (213 cm).

Number of primary branches per plant: The number of primary branches per plant varied from 11 to 22. Among the CMS lines, only AL 117A (22) recorded significantly more value than GT 288A for this trait. Nine out of 20 CMS lines were statistically at par with GT 288A (17) whereas, ten CMS lines were statistically at par with ICP 2089A (16) for primary branches per plant.

Number of secondary branches per plant: Three out of 18 CMS lines namely, AL 120A (28), AL 103A (26) and

AL 117A(23) were statistically at par with GT288A(25) and had significantly higher number of secondary branches per plant than ICP 2089A whereas, ten CMS lines were statistically at par with ICP 2089A (20). The CMS line AL 120A (28) exhibited maximum value for number of secondary branches per plant followed by AL 103A (26) and GT 288A (25).

Number of pods per plant: Increased number of pods per plant results into significant improvement in the yield. Sufficient variability was observed for number of pods per plant. The range for this trait was from 88 to 260. AL 120A (260) exhibited maximum number of pods per plant followed by AL 103A (256). These two CMS lines had significantly higher number of pods per plant than GT 288A (204).

Number of seeds per pod: The number of seeds per pod ranged from 3.3 to 3.9. The CMS lines AL 103A, AL 120A) recorded maximum value (3.9) and were statistically at par with GT 288A for number of seeds per pod and two CMS lines namely, AL 114A and AL 117A (3.8) were statistically at par with ICP 2089A.

100-seed weight (g): The 100-seed weight was ranged from 6.9 g to 8.5 g with mean value of 7.8 g. Eight CMS lines namely, AL 102A (8.0 g), AL 103A (8.1 g), AL 105A(8.1 g),AL112A(8.1 g),AL114A(8.0 g),AL115A(8.1 g), AL117A(8.1 g) and AL 120A (8.3 g) were statistically at par with ICPA 2089A whereas, AL120A (8.3 g) which was at par with GT 288A (8.5 g) was significantly superior for 100-seed weight than ICP 2089A (8.1 g).

Grain yield per plant (g): Grain yield per plant varied from 21.4 g (AL 118A) to 61.9 g (AL 120A). Six CMS lines namely, AL120A(61.9 g), AL103A(61.7 g), AL100A(52.2 g), AL111A(52.0 g), AL115A(50.4 g), AL117A(55.2 g), showed significantly higher grain yield than ICP 2089A (40.1 g) whereas, CMS lines AL120A(61.9 g) and AL 103A (61.7 g) were higher yielding than GT288A (58.3 g).

Therefore, the present investigations revealed important information about the CMS lines that these lines are early maturing and stable and hence can be used in development of early maturing hybrids suitable in pigeonpea wheat rotation in north India including Punjab. These stable male sterile lines will also reduce the cost of hybrid seed production by eliminating artificial emasculation. This, in turn, will help to increase area under pigeonpea crop.

REFERENCES

Gowda MB and Basha SH. 2005. Evaluation of new cytoplasmic and environmental sensitive male sterile lines of pigeonpea. IFLRC IV, Oct. 2009, New Delhi, India. Pp.152.

PAU. 2013. Package of Practices for Kharif crops, Punjab Agricultural University (PAU), Ludhiana. Pp.66.

Saxena KB. 2009. Evaluation of hybrid breeding technology in pigeonpea. In: Masood Ali and Kumar S (Eds) Advances in Food Legume Research Pp. 82-114, IIPR, Kanpur, India.

Saxena KB. 2013. A novel source of CMS in pigeonpea derived from *Cajanus reticulatus*. Indian Journal of Genetics 73:259-63.

Saxena KB and Kumar RV. 2003. Development of a cytoplasmic-nuclear male sterility system in pigeonpea using *C. scarabaeoides* (L.) Thouars. Indian Journal of Genetics 63:225-29.

Saxena KB, Chauhan YS, Johansen C and Singh L. 1992. Recent developments in hybrid pigeonpea research. In: Proc Workshop on New Frontiers in Pulses Research and Development Pp.58-69.

Saxena KB, Kumar RV, Dalvi VA, Pandey LB and Gaddikeri G. 2010. Development of cytoplasmic-nuclear male sterility, its inheritance and potential use in hybrid pigeonpea breeding. Journal of Heredity 101:497-503.

Saxena KB, Kumar RV, Madhavi LK and Dalvi VA. 2006. Commercial pigeonpea hybrids are just a few steps away. Indian Journal of Pulses Research 19:7-16.

Singh NB, Singh IP and Singh BB. 2005. Pigeonpea breeding. In: Masood Ali and Kumar S (Eds) Advances in Pigeonpea Research. IIPR, Kanpur, India. Pp.67-95.

Tikka SBS, Parmar LD and Chauhan RM. 1997. First record of cytoplasmic-genic male sterility system in pigeonpea (*Cajanus cajan* (L.) Millsp.) through wide hybridization. Gujarat Agriculture University Research Journal 22:160-62.

Verma MM and Sidhu PS. 1995. Pigeonpea hybrids: historical development, present status and future prospective in Indian context. In: M Rai and S Mauria (Eds) Hybrid Research and Development. Indian Society of Seed Technology, IARI, New Delhi, India. Pp.121-37.

Wanjari KB and Rathod ST. 2012. Exploitation of heterosis through F_1 hybrid in pigeonpea (*Cajanus cajan* L.) the status and prospects. Indian Journal of Genetics and Plant Breeding 72:257-63.

Wanjari KB, Patil AN, Manjaya JG and Patel MC. 1999. AKPH-4101: A short duration pigeonpea hybrid for Central Zone of India. International Chickpea and Pigeonpea Newsletter 6:39-40.

Zhang BX Wang LH and Guo JZH. 2002. Investigation of male fertility of pepper in CMS. China Capsicum 4:6-9.