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Original Research Paper

Stability of cytoplasmic male-genic sterility in pigeon pea (*Cajanus cajan* (L.) Millsp.) under different environmental conditions in Kenya

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In pigeon pea (*Cajanus cajan* (L) Millsp.), a stable cytoplasmic-genic male sterile system (CMS) of A4 cytoplasm derived from a cross between cultivated and wild relative was developed that opened up the possibility of production of commercial hybrids. Promising stable CMS lines have been developed in India and the hybrids produced with these lines have high heterosis. The use of highly stable CMS will reduce the cost of hybrid seed production by eliminating the task of emasculation. The aim of this study was to investigate the stability of pollen sterility and morphological characteristics of several CMS lines under Kenyan conditions. Three sites; Katumani (1°35'S, 37°14'E; 1,600 m), Kiboko (2°15'S, 37°45E; 960 m), and Leldet-Nakuru (0°31'E, 0°,09'S; 1,275 m) were selected for evaluation. Six CMS lines, with over 96% cytoplasmic male sterility, and their maintainers were sourced from ICRISAT India and evaluated for two seasons in 2009 in a screen house at Katumani and Kiboko and in an isolated field at Leldet Nakuru. Two CMS lines, ICPA2043 and ICPA2039 were the most stable across sites with 100 and 99% pollen sterility, respectively. Days to flower showed 2 to 11-day variations between the A- and B-lines, but were not significantly different. Performance of the two promising CMS lines under Kenyan conditions for pollen sterility was comparable to the results obtained in India and can, therefore, be used in commercial hybrid breeding.

Key words: Cytoplasmic male sterility, pigeon pea, fertility stability.

INTRODUCTION

In Kenya, pigeon pea [*Cajanus cajan* (L) Millsp.] is the third most important food grain legume after common bean (*Phaseolus vulgaris* L.) and cowpea (*Vigna unguiculata* L.) (Kimani et al., 1994; Mergeai et al., 2001; Jones et al., 2002). It is adapted to a range of environments with varying temperatures, altitudes and latitudes (Troedson et al., 1990; Silim et al., 2006). The crop is cultivated by smallholder farmers in the arid and semi-arid regions of Eastern Kenya where it is commonly intercropped with other legumes, cereals and fruit trees. However, the yields remain low, ranging between 500 to 800 kg ha^{-1} as compared to a potential of >3000 kg ha^{-1} (Omanga et al., 1995).

Pigeon pea improvement research in Kenya was initiated in the mid-1970s with breeding activities centred on collection, evaluation and selection. Adoption of improved varieties released by national and international research institutions is evident in many parts of the Eastern Province (Sutherland et al., 1999; Jones et al., 2001). Recently pigeon pea hybrids have been successfully developed by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) for India and this technology could be of importance for future pigeon pea breeding in Kenya. One of the first steps is to study the stability of the cytoplasmic-genic male sterility (CMS) in pigeon peas under Kenyan conditions.

In pigeon pea, several stable CMS systems have been developed (Saxena and Kumar, 2003; Mallikarjuna and Saxena, 2005; Saxena et al. 2005). The line ICPW29, an accession of *Cajanus cajanifolius* (Haines) van der Maesen) (A4 cytoplasm), a wild relative of pigeon pea was crossed with cultivated type and after seven backcrosses, developed the CMS line ICPA2039. It was found to be a highly stable male sterile line across environments and years and never

showed any morphological deformity (Dalvi et al., 2008; Saxena et al., 2010b). The CMS line ICPA2039 has been used to develop other CMS lines with resistance to diseases, with various maturity periods and with adaption to diverse environments of India (Saxena, 2008).

Environmental conditions are known to influence the expression of nuclear and cytoplasmic male sterility genes in some crops, whereby sterility and fertility changes depend on day length and/or temperature (Janska and Mackenzie, 1993). Ariyanayagam et al. (1995), using sensitive pigeon pea genotypes, established that short day length and low temperatures induce male fertility, while high temperatures and longer days maintain male sterility. In CMS cotton (*Gossypium* spp.), wind velocity, air temperature, global radiation, and pan evaporation have been shown to influence expression of male sterility two to three weeks before anthesis (Marshall et al., 1974; Weider et al., 2009). In rape seed (*Brassica napus* L.), day-night temperatures of 22 to 16^{o}C resulted in stability of sterility while day-night temperatures of 30 to 24^{o}C promoted anther development (Fan and Stefansson, 1986). On the other hand, CMS onion (*Allium cepa* L.) had more mature pollen at low temperature (Peterson and Foskett, 1953).

As in any breeding programme for pigeon pea, a desirable CMS line should possess yield components that have a direct positive effect on yield. These include; days to 50% flowering, primary branches, plant height, number of pods per plant and seed mass (Lal and Raina, 2002; Egbe and Vange, 2008; Bhadru, 2010). Favourable yield components will enable availability of sufficient number of flowers for pollination hence high seed yields.

The objective of this study was to evaluate several cytoplasmic male sterile lines of Indian origin for stability across several environments in Kenya.

MATERIALS AND METHODS

Experimental sites

The study was conducted at three sites; Katumani, Kiboko and Leldet farm-Nakuru.

Katumani lies at 1^{o}35'S and 37^{o}14'E at 1600 m above sea level (masl). The centre experiences a semi-arid tropical climate in AEZ IV with a bimodal pattern of rainfall. The first rains come in March with a peak in April followed by a dry period extending from June to mid-October. The short rains occur from mid-October with a peak in November and taper off towards mid-December. Long rains amount to 272 mm and short rains 382 mm. Mean maximum temperature is 24.7°C and mean minimum is 13.7°C.

Kiboko lies at 37°45'E and 2°15'S with an elevation of 960 masl. It is characterized by high temperatures with a mean minimum and maximum of 16.9 and 31°C respectively. It has a bimodal pattern of rainfall ranging between 200 to 400 mm per season.

Leldet lies at 0°31'E and 0°09'S with an altitude of 2,275 masl on the outskirts of Nakuru. The area experiences bimodal pattern of rainfall which are erratic with peaks in April and August and an annual mean of 380 mm. The area can be hot with mean maximum temperatures of 26°C and a mean minimum of 14°C, with the warmest months being November to February.

Genotypes

The experimental materials comprised of six CMS lines (ICPA2043, ICPA2039, ICPA2091, ICPA2050, ICPA2042 and ICPA2101) with A4 cytoplasm and their corresponding maintainers (ICPB2043, ICPB2039, ICPB2091, ICPB2050, ICPB2042 and ICPB2101). These were obtained from ICRISAT India where they had been developed by manual hand pollination under cages. To protect the experimental materials from pollinating insects, the seeds were planted inside nylon net with pore size of 0.5 mm screen house at the Kenya Agricultural Research Institute (KARI) in Kiboko and Katumani.

At Leldet Farm in Nakuru the A- and B-lines were planted in an open field, but at an isolation distance of 500 m to other pigeon peas as recommended by Saxena et al. (2005). Each line was planted in 9.5 m^2 plot of two rows spaced at 100 cm × 50 cm inter- and intra-row in a randomized complete block design with two replications. Twenty seeds were planted per plot with one seed per hill. Manual weeding using a hand-hoe was done regularly. Dimethoate (Duduthrin) was used as a standard commercial insecticide. For the management of red spider mites, acaricide sprays were done after scouting on the plants. The first spray was applied at flower bud expansion stage and subsequent sprays at 14-day intervals.

Individual plants of the CMS lines were examined for male sterility with B-lines as controls. Male sterility was assessed by sampling 10 fully grown but unopened flower buds from 20 plants. Initial examination was done by rubbing anthers between fingers and a 10× hand lens was used to establish the presence or absence of pollen. Further analysis was done by squashing anthers in 2% aceto-carmine stain (Zhang et al., 2002) and examination done under the microscope using a haemocytometer. In each slide, three microscopic fields were examined and counts were made for male-sterile (shrivelled and unstained) and male fertile (round and red colour stained) pollen grains. The counts were converted to percentage sterility. The relative amount of non-viable pollen was used to classify the CMS lines. For morphological characterization, in each set, 10 plants were randomly selected and data were recorded on days to 50% flowering, days to 75% maturity, plant height (cm), and number of primary branches, growth habit, seed mass and seeds pod^{-1}. Days to maturity, seeds pod^{-1}, and seed mass (100-seed weight) were estimated based on the B-lines only.

Figure 1. A-Sterile anthers, (ICPA2043) B- fertile anthers with viable pollen (ICPB2043) of pigeon pea flowers at Kiboko, Kenya.

Table 1. Mean square from combined analysis of variance for number of primary branches, days to flower and plant height on cms (a) and b-lines of pigeon pea at Katumani, Kiboko and Leldet in 2009.

Source	df	Number of primary branches	Days to flower	Mean squares of plant height (cm)
Rep	1	16.6	149.5	90.4
Genotype	11	94.7***	6706.4***	13190.7***
Site	2	185.4***	11616.3***	63.1
Season	1	12.8*	1.9	1894.4**
Genotype × Site	22	3.9	1130***	628.1***
Genotype × Season	11	2.3	143.9	341.8
Site × Season	2	132.6***	427.6	717.6*
Genotype × Site × Season	22	13.3***	188.7	409.8**
Error	71	1.6	153.5	187.1

*, **, ***significant at P≤0.05, P≤0.01, and P≤0.001, respectively.

Data analysis

Statistical analyses were done using GENSTAT 14th edition (Payne et al., 2011) statistical programme using the following statistical model:

$$Y_{ijklm} = \mu + l_m + a_i + (al)_{im} + t_k + (tl)_{km} + (at)_{ik} + (atl)_{ikm} + g_{ij} + s_{ijk} + (gl)_{ijm} + (sl)_{ijkm} + \varepsilon_{ijklm}$$

Where: μ = overall mean, l_m = effect of the mth location, a_i = effect of the ith experiment, t_k = effect of the kth tester; g_{ij} = gca effect Line j in Experiment I, s_{ijk} = sca effect of Line j in Experiment I with Tester k, and corresponding interaction effects with locations, and ε_{ijklm} = averaged plot residual. Analysis of variance was determined using Residual Maximum Likelihood (REML).

Stability patterns of the parameters were measured using regression coefficient (b) between the genotypic mean values and the environmental mean values (Finlay and Wilkinson, 1963). This allowed for estimation of general combining ability (GCA) and specific combining ability (SCA) effects. Means were separated using protected Fisher test of LSD.

RESULTS

From the combined analysis of variance across sites, genotypes and genotype × site interactions were significantly different for all the traits recorded (Table 1). Days to flower and plant heights were significantly different (P≤0.001, P≤0.01 and P≤0.05) for genotypes, sites, and genotype × site interaction. From the mean squares for B-lines for days to maturity, seed mass and seeds/ pod, days to maturity was significantly different among genotypes, sites and site × season interactions (Table 2). Means for seeds/pod, seed mass were significantly different for genotypes, season and their interactions.

When means were compared across sites, the mean for days to flower was 100 days and ranged from 77 days (ICPA2039) to 152 days to flower (ICPA2091) (Table 3).

Table 2. Mean square combined analysis of variance for days to maturity, seed mass and seeds/pod on cms (b) lines of pigeon pea at Katumani, Kiboko and Leldet in 2009 .

Source	df	Mean squares		
		Days to maturity	Seed mass (g)	Seeds/pod
Rep	1	4480.9	0.6	0.5
Genotype	5	24233.4***	166.7***	5.7***
Site	2	10271.3***	3.4	2.5**
Season	1	1088.9	19.2***	0.9*
Genotype × Site	10	1162.0	12.5*	1.2***
Genotype × Season	5	822.9	35.1**	1.7**
Site × Season	2	2996.5**	12.3*	1.9*
Genotype × Site × Season	10	496.0	12.1***	1.7***
Error	35	426.5	0.7	0.2

*, **, ***significant at P≤0.05, P≤0.01, and P≤0.001, respectively.

Table 3. Means of agronomic traits recorded on pigeon pea cytoplasmic male sterile A- and B-lines across sites and seasons.

Genotype	Number of primary branches	Days to flower	Days to maturity	Seed mass (g)	Plant height (cm)	Pollen sterility (%)	Seeds pod^{-1}
ICPA2039	6.9	80.0	-	-	69.0	99.0	-
ICPA2042	6.1	93.0	-	-	92.0	5.0	-
ICPA2043	9.7	96.0	-	-	119.0	100.0	-
ICPA2050	9.5	98.0	-	-	116.0	53.0	-
ICPA2091	14.2	146.0	-	-	170.0	71.0	-
ICPA2101	6.7	91.0	-	-	77.0	39.0	-
ICPB2039	6.3	77.0	136.0	9.5	72.0	0	4.0
ICPB2042	5.3	90.0	172.0	5.4	90.0	0	2.8
ICPB2043	9.3	94.0	165.0	11.2	120.0	0	4.0
ICPB2050	8.5	100.0	199.0	14.9	111.0	0	4.8
ICPB2091	12.6	152.0	269.0	5.2	159.0	0	3.9
ICPB2101	5.7	88.0	184.0	10.4	75.0	0	4.7
Mean	8.4	100.0	188.0	9.4	106.0	31.0	4.0
LSD$_{(0.05)}$	2.5	25.0	46.0	1.6	27.0	5.0	0.9
CV (%)	15.0	12.0	12.0	8.7	13.0	9.0	10.6

ICPA2039 (99%), which was also significantly different from the other genotypes. The two A-lines recorded the highest sterility across sites and seasons. Pollen sterility for A-lines ranged from 5 to 100%. The A-lines, ICPA2043 and ICPA 2039 recorded 100 and 99% sterility across sites and seasons. The mean for plant height was 106 cm, but showed variation among the genotypes ranging from 69 (ICPA2039) to 170 cm (ICPA2091).

The A- and B-lines of ICPA2043 produced an average of 10 branches per plant while ICPA2039 produced 7 branches. Days to flower did not vary markedly between A- and B-lines. All genotypes showed delayed flowering at Katumani in the first season as compared to other sites with a mean of 100 days. ICPB2039 flowered earliest (77 days) while ICPA2091 flowered latest (152 days). The difference in days to flower between ICPA2043 and the corresponding B- line was 2 days. A t-test analysis to compare days to flower for A-and B-lines was not significantly different (t-stat of 0.04), with A-lines having a mean of 101 and B-lines, 100 days. The mean for days to maturity was 188 days and ranged between 269 days (ICPB2091) and 136 days (ICPB2039). The B-lines at Katumani in the first season took more days to maturity (221) as compared to 162 days at Kiboko. ICPB2039 matured earliest at Katumani (161 days), but ICPB2043 matured at 193 days. Overall, mean seed mass was 9 g across sites. The highest seed mass was recorded on ICPB2050 (14.9 g) and the lowest was for ICPB2042 (5.4 g) and ICPB2091 (5.2 g). Seed mass was consistent across sites.

Male sterile and fertile anthers were observed on both A- and B-lines (Figure 1). The extent of male sterility among

Table 4. Means for pollen sterility, days to flower and days to maturity across sites and seasons.

Genotype	Pollen sterility (%)						Days to flower						Plant height (cm)					
	Katumani		Kiboko		Leldet		Katumani		Kiboko		Leldet		Katumani		Kiboko		Leldet	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
ICPA2039	100	100	96	100	100	100	86	100	73	71	72	77	77	82	62	64	61	66
ICPA2042	2	2	21	1	2	3	123	118	83	70	88	79	86	94	97	101	77	95
ICPA2043	100	100	100	100	100	100	107	123	91	77	89	88	123	129	104	156	102	99
ICPA2050	67	56	46	62	47	41	104	122	74	86	112	92	94	131	118	98	112	140
ICPA2091	77	69	45	77	75	81	175	190	123	134	134	121	147	193	189	167	153	168
ICPA2101	14	38	80	17	35	50	77	72	81	88	107	121	69	63	63	64	119	86
ICPB2039	0	0	0	0	0	0	84	97	75	68	74	67	77	84	68	69	63	71
ICPB2042	0	0	0	0	0	0	127	104	65	70	85	88	77	95	101	95	83	94
ICPB2043	0	0	0	0	0	0	119	107	90	79	89	81	119	129	116	150	108	99
ICPB2050	0	0	0	0	0	0	117	114	74	90	116	87	93	119	98	111	113	132
ICPB2091	0	0	0	0	0	0	185	218	121	139	130	119	139	184	172	165	150	143
ICPB2101	0	0	0	0	0	0	78	75	80	86	107	106	71	61	71	69	106	74
LSD($_{0.05}$)	5						25						27					
CV (%)	9						12						13					

1=2009 growing season, 2=2010 growing season.

Table 5. Means for days to maturity, 100-seed weight and seeds per pod across sites and seasons at katumani, kiboko and leldet.

Genotype	Days to maturity						Seed mass (100-seed weight)						Seeds pod^{-1}					
	Katumani		Kiboko		Leldet		Katumani		Kiboko		Leldet		Katumani		Kiboko		Leldet	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
CPB2039	161	157	104	118	131	147	10	10	7	9	10	10	4	4	4	4	4	4
ICPB2042	202	170	164	160	168	168	10	2	7	7	8	3	3	2	3	3	4	3
ICPB2043	193	181	135	187	144	154	13	12	10	11	9	13	4	4	4	4	4	4
ICPB2050	221	190	173	163	225	226	16	18	13	14	13	16	5	5	5	5	5	5
ICPB2091	309	244	250	216	280	314	7	6	8	8	7	7	5	5	4	5	5	5
ICPB2101	240	195	176	133	172	188	10	10	12	8	9	13	4	5	4	5	5	5
Mean	221	189	167	162	187	199	11	8	9	10	9	9	4	3	4	4	4	4
LSD($_{0.05}$)	42						2						1					
CV (%)	11						9						11					

1=2009 growing season, 2=2010 growing season.

Table 6. Regression coefficients of some morphological traits of cytoplasmic male sterile pigeon pea lines and their maintainers across sites and seasons.

Genotype	Morphological traits				
	Days to flower	Days to maturity	Number of branches	Seed mass (g)	Plant height (cm)
ICPA2039	1.1	-	1.1	-	0.1
ICPA2042	0.6	-	0.6	-	0.3
ICPA2043	0.8	-	0.7	-	0.1
ICPA2050	0.7	-	0.4	-	0.2
ICPA2091	0.5	-	0.6	-	0.2
ICPA2101	0.3	-	1.5	-	0.1
ICPB2039	1.1	0.7	0.9	0.2	0.2
ICPB2042	0.6	0.9	0.7	0.2	0.4
ICPB2043	0.8	0.2	0.8	0.1	0.1
ICPB2050	0.7	0.5	0.5	0.1	0.2
ICPB2091	0.3	0.4	0.6	0.2	0.2
ICPB2101	0.4	0.4	1.2	0.2	0.1

the A-lines ranged between 5 and 100%. The highest percentage was observed on ICPA2043 (100%) and for ICPA 2050 and ICPA2091 was between 41 and 81%). ICPA2042 recorded less than 5% pollen sterility, except during the first season at Kiboko where 21% was recorded. Pollen sterility on ICPA2101 varied between 14 and 80% but without showing any particular trend.

The observed results for number of primary branches, days to flower and plant height on A-lines and days to maturity, seed mass and seeds pod^{-1} on B-lines showed that the growth habit and environments had a direct influence on plant height. Significant differences were recorded between genotypes, genotype × site and genotype × season interactions (Tables 4 and 5). ICPA2039 and ICPA2101 were determinate and therefore gave the shortest height. The tallest genotype across locations was ICPA2091 (170 cm) and the shortest was ICPA2039 (69 cm). Across environments, Katumani in the second season had the highest mean plant height (114 cm), but lowest (98 cm) in the first season. Mean number of branches per plant varied among the genotypes. The highest number of branches were recorded on ICPA2091 (14.2) and lowest on ICPA2042 (5.3).

Regression coefficients for some of the morphological characteristics studied described the stability of the response of pigeon pea genotypes grown in several locations (Table 6). Regression coefficients for days to flower was recorded for ICPA2039 (1.1), ICPB2039 (1.1), ICPA2043 (0.8), and ICPB2043 (0.8). ICPA2039 and ICPA2043 gave between 96 and 100% sterility across sites and seasons. Number of primary branches recorded a regression coefficient of > 1.0 for ICPA2039 and ICPA2101.

Conclusion

The study was aimed at determining the most suitable CMS lines for use in the pigeon pea breeding programmes in Kenya. Highly significant differences between the genotypes and sites for most characters revealed that considerable variability exists amongst the CMS lines and the maintainers in different seasons for the traits recorded (Sharma and Green (1977; Sidhu et al., 1985). There is therefore need to categorize genotypes for their adaptation to varying environmental conditions using their respective regression coefficient values as suggested by Finlay and Wilkinson (1963). The recommended levels of male sterility (>95%) (Saxena et al., 2005) was recorded on ICPA2043 and ICPA2039 across all sites and seasons. The two A-lines could be used for seed production in different environments in Kenya.

Days to maturity of the A- and B- lines shows that they belong to the early and medium maturity groups. There is therefore the potential to utilize these A-lines to develop medium maturity hybrids. Similar work has been done in India (Saxena et al., 2010b), where relatively late flowering CMS were used in breeding long- and medium-maturity hybrids for specific environments. Days to flowering for the promising CMS lines and their maintainers were not significantly different. This indicates that the A- and B-lines can be planted at the same time without affecting seed production.

The number of primary branches indirectly influences seed yield through the number of pods plant^{-1} (Lal and Raina, 2002). On average ICPA2043 and ICPA2039 produced 10 and 7 branches, respectively. The potential for ICPA2043 to produce 21 branches and ICPA2039 (15) has been recorded on individual plants in the trial plots (Saxena et al., 2005). The differences were expected as ICPA2043 is non-determinate but ICPA2039 is determinate.

Commercial use of CMS requires highly stable male sterility, to ensure genetically pure F1 hybrid seed. The CMS lines ICPA2043 and ICPA2039 were highly stable and

recorded mean pollen sterility of 100% and 99% respectively across the locations and seasons. The stable sterility of these lines was also reported by Dalvi et al. (2008) and Saxena (2008) who found high stability for pollen sterility across environments in India. The two CMS-lines were therefore suitable for use in commercial hybrid breeding programmes in that country (Saxena, 2008; Saxena et al., 2010a).

Performance of the two CMS lines under Kenyan conditions for pollen sterility was comparable to that in India. The most preferred sites for seed production are those with optimal growth conditions for pigeon pea. In this study, Kiboko, where irrigation can be used was the ideal site as plants produced more branches and were taller indicating high vigour that is likely to lead to high seed yields. The study has shown that commercial hybrid seed production in Kenya is feasible if male parents are carefully selected and the F_1 hybrids are tested across different environments for yield.

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