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Evaluation of CMS Lines and Its Maintainers in Pigeonpea (*Cajanus cajan* L. Millsp.)

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ABSTRACT
Ten lines [Five male sterile lines (A) and five maintainer lines (B)] of pigeonpea [Cajanus cajan (L.) Millsp.] were subjected to investigation and evaluation for their quantitative (12) and qualitative (10) attributes under Ranchi condition at GVT Pulse Farm, Kanke, Ranchi, Jharkhand during Kharif 2012-13. The observations were made for twelve quantitative and ten qualitative characters. Significant differences were observed among the lines for all the traits; indicating the presence of efficient variability among the male sterile lines and the maintainer lines. On the basis of average pod setting in bagged plant of male sterile lines, the highest number of pods was found for ICPA 2078 (8 pods) and lowest for ICPA 2092 (Zero pod).The pod setting value is very low or negligible; suggesting that all male sterile lines performed well in Ranchi condition particularly ICPA 2092(A_4) followed by ICPA 2047-24(A_3) and ICPA 2051(A_5).The survival percentage of all male sterile lines (A) was observed more in comparison to its maintainer lines. On the basis of pollen sterility and fertility test, the maximum pollen sterility was observed for the line ICPA 2078(A_2) whereas lowest pollen sterility was found ICPA 2092(A_4) line, although all male sterile lines were found suitable for Ranchi condition. The pollen fertility of maintainer lines were observed maximum for ICPB 2047-24(B_3) and lowest for ICPB 2043(B_1). The result obtained from present investigation indicated that all the A lines were found better but ICPA 2047-24(A_3) was found best on the basis of observation of field as well as lab test. All were found at par as compared to A and B lines. Male sterile cytoplasm is affecting germination percentage in all the CMS line in positive direction. Male sterile cytoplasm has effect on plant's survival in desired direction i.e., high survival % in CMS line as compared to maintainer line. In the present investigation, male sterile cytoplasm was not affected any of the qualitative traits. However, male sterile cytoplasm has significant affect on quantitative traits.
Key words: Pigeonpea, Maintainer lines, CMS lines, Ranchi

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INTRODUCTION

Pigeonpea [*Cajanus cajan* (L.) Millspaugh], is a short-lived perennial member of family Fabaceae and it is invariably cultivated as annual crop. Pigeonpea is an often cross-pollinated (20–70 percent) crop with 2n=2x=22 diploid chromosome number. Pigeonpea is a hardy, widely adapted and drought tolerant crop. Recently, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) developed a super early genotype maturing in 70-75 days. The super early (<100 days) and short-duration (100-140 days) cultivars are grown as sole crop, whereas in Jharkhand, early pigeonpea varieties grown as intercrop with millet, maize, groundnut, *etc.* and medium & long duration pigeonpea grown as sole crop. The development of commercial hybrid pigeonpea program was innovated at ICRISAT in collaboration with ICAR (Indian Council of Agricultural Research). In 1974, a source of genetic male-sterility (GMS) was identified. As a consequence, a genetic male-sterility based pigeonpea hybrid ICPH 8 was released in 1991 in India (Saxena *et al*., 1992a). This development provided the most important information on the role of partial natural out-crossing in large-scale hybrid seed production. This component is essential for commercial exploitation of hybrid vigour in pigeonpea (Saxena and Nadarajan, 2010). Natural out-crossing in pigeonpea was first reported by Howard *et al*. (1919). The out-crossing in this crop is mediated by a variety of insects (Onim, 1981) and wind does not play any role in this event. The estimates

of natural out-crossing vary greatly between 2-70 percent in different three environmental conditions (Saxena *et al*., 1990). In pigeonpea, several stable CMS systems have been developed (Saxena and Kumar, 2003; Mallikarjuna and Saxena, 2005; Saxena *et al*. 2005). The CMS line ICPA 2039 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. This study was designed for proper understanding of underlying inheritance of quantitative and qualitative traits and also in identifying the promising CMS lines for further use in hybrid seed production. In view of above consideration, the present study was planned for development of location specific new hybrids, we needed stable cytoplasmic male sterile and its maintainer line for Ranchi location.

MATERIALS AND METHODS

The experimental material comprised of five male sterile lines *viz*. ICPA 2043(A_1), ICPA 2078(A_2), ICPA 2047-24 (A_3), ICPA 2092 (A_4) and ICPA 2051 (A_5) and five maintainer lines *viz*. ICPB 2043 (B_1), ICPB 2078(B_2), ICPB 2047-24 (B_3), ICPB 2092(B_4) and ICPB 2051(B_5). These materials obtained from International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru. All these materials were evaluated at Ranchi location. The present study was carried out in the GVT Pulse Farm, Birsa Agricultural University, Kanke, Ranchi during *Kharif* 2012-13. The CMS and maintainer lines were evaluated in a paired t-test design. The experiment was sown in 21st July, 2012. The plot size was of 18 m^2. The plot divided into 5 small blocks in which 8 lines were placed in each block. The row length was 3m and 30 x 75 (cm) spacing was maintained between plant to plant and row to row. The seeds treated with Bavistin to control the seed borne diseases followed by Rhizobium and PSB (Phosphate Solubilizing Bacteria) culture. Two days after sowing (DAS) one sprays of Pendimethalin @ 4ml/lit. applied to control weeds, whereas 30 days after sowing (DAS), one spray of Quizalophop ethyl 5 per cent EC (Targa Super) @ 2ml/lit. applied as a post emergence weedicide. During the reproductive period, there was serious damage due to pod borers (*Maruca testulalis & Helicoverpa armigera*) and termite. Therefore one spray of Chlorpyriphos was applied @ 2ml/lit whereas Indoxacarb @ 0.5ml/litre followed by monocrotophos @ 2ml/litre of water were used to control pod borers.

For testing the pollen fertility and pollen sterility in the maintainer line & CMS lines, 2 percent aceto-carmine solutions was used to stain and differentiate the fertile and sterile pollen grains. Five plants were selected randomly from each CMS line and maintainer line and five buds from each plant were collected to record its pollen fertility & pollen sterility. Anthers from each flower bud were squashed on a slide and the count of fertile and sterile pollen grains in three microscopic fields was noted. Statistical analysis was done and for pollen fertility and percent pollen sterility was calculated by following formula:

Pollen fertility (percent) = $\frac{FP}{TP} x100$

Pollen sterility (percent) = $\frac{SP}{TP} x100$

Where; FP- number of fertile pollen, SP- Number of sterile pollen, TP- Total number of pollen grains.

When there are two sets of data in which the observations are paired, we take the differences of these paired observations and then considering these differences as a small sample of differences, compare the mean difference with zero, under the assumption that there is no difference between the observations of any of the different pairs.

RESULTS AND DISCUSSION

The mean value and fisher's t-value were calculated for seven quantitative characters have been presented in Table 1. It was revealed from the table that among all A and B lines, we were found significant difference for the some characters among all A and B lines. The average performance of ICPA 2043 (A_1) and ICPB 2043 (B_1) lines were found significantly different for the character *viz.,* number of secondary branches (27.2, 22.0) and number of pods per plant (181.6, 162.8) and high magnitude for A line. However for the character i.e. days to 1st flowering (107.8, 105.8), days to 50 percent flowering (113.6, 112.2), number of primary branches (13.9, 13.2), plant height (136.4, 131.1), and days to maturity (220, 221), the average performance of both lines were not differed significantly but again high magnitude were found for A_1 line .The average performance was found non-significant for the character viz., days to 1st flowering (93.1, 96.1), days to 50 percent flowering (99.4, 103.1), number of primary branches (17.7, 18.4), plant height (104.2, 104.0), and days to maturity (212, 214.2) and on the other hand, for the character number of secondary branches (25.7, 28.2) and number of pods per plant(88.6,

100.9) of A_2 & B_2 were found significantly different but magnitude of B_2 lines were found high .For the character viz., days to 1st flowering (108.6, 110.9), number of primary branches (14.9, 10.2), number of secondary branches (24.9, 22.6), number of pods per plant (182.9, 147.9), the average performance of ICPA 2047-24(A_3) & ICPB 2047-24 (B_3) were found significantly differed and high magnitude of A_3 was observed whereas for these two lines, non-significant mean performance were observed for the character viz., days to 50 per cent flowering (115.4, 116.8), plant height (166.7, 168.6) and days to maturity(220.9, 222.0) and found high magnitude of B_3 line. The mean performance of ICPA 2051 and ICPB 2051 were found significant for the characters *viz.,* days to 1st flowering (109.2, 113.0), days to 50 per cent flowering (118.8, 124.0) but found high magnitude of mean for B_5 line, and for the character number of pods per plant (183.6, 125) was also found significant but high magnitude for A_5 was observed. However these two lines also showed non-significant for the characters viz. number of primary branches (19.2, 18.2), number of secondary branches (27.1, 25.2), plant height (166.9, 165) and days to maturity (223.5, 224.0) but observed high magnitude for A_5 line. The paired t-value of the A and B lines for different quantitative characters are presented in Table 2.

Table -1: Mean of different traits of A and B lines of pigeonpea

Lines/Parameters	ICPA 2043 (A_1)	ICPB 2043 (B_1)	t-value (fisher)	ICPA 2078 (A_2)	ICPB 2078 (B_2)	t- value (fisher)	ICPA 2047-24 (A_3)	ICPB 2047-24 (B_3)	t- value (fisher)	ICPA 2092 (A_4)	ICPB 2092 (B_4)	t- value (fisher)	ICPA 2051 (A_5)	ICPB 2051 (B_5)	t- value (fisher)
	Mean	Mean		Mean	Mean		Mean	Mean		Mean	Mean		Mean	Mean	
Days to 1st flowering	107.8	105.8	NS	93.1	96.12	NS	108.6	110.96	*	115.1	93.8	**	109.2	113	**
Days to 50per cent flowering	113.6	112.2	NS	99.4	103.1	NS	115.4	116.84	NS	123.3	100	**	118.8	124	**
No. of Primary Branches	13.9	13.2	NS	17.7	18.4	NS	14.9	10.2	**	14.2	13.1	NS	19.2	18.2	NS
No. of Secondary Branches	27.2	22.0	**	25.7	28.2	**	24.9	22.6	**	25.5	23.4	NS	27.1	25.2	NS
Plant height (cm)	136.4	131.1	NS	104.2	104	NS	166.7	168.6	NS	137.5	136	NS	166.9	165	NS
No. of Pods/plant without bag	181.6	162.8	**	88.6	100.9	**	182.9	147.96	**	107.5	93.3	NS	183.6	125	**
Days to maturity	220	221	NS	212	214.2	NS	220.9	222	NS	227	225	NS	223.5	224	NS

* Significant at 5per cent level ,**Significant at 1per cent level & NS- Non-significant

Table-2: Paired t-value for characters evaluated in pigeonpea

Lines/Parameters	Paired t-value (A_1B_1)	Paired t-value (A_2B_2)	Paired t-value (A_3B_3)	Paired t-value (A_4B_4)	Paired t-value (A_5B_5)
Days to 1st flowering	NS	NS	*	**	**
Days to 50per cent flowering	NS	NS	NS	**	**
No.of Primary Branches	NS	NS	**	NS	NS
No. of Secondary Branches	**	**	**	**	*
Plant height (cm)	NS	NS	NS	NS	NS
No.of Pods/plant without bag	**	*	**	**	**
Days to maturity	NS	NS	NS	NS	NS

* Significant at 5per cent level, **Significant at 1per cent level & NS- Non-significant

The pod setting value is negligible. The highest was found for ICPA 2078 (8 pods) followed by ICPA 2043 (6 pods), ICPA 2047-24 (2 pods), ICPA 2051 (2 pods) and lowest for ICPA 2092 (zero pod) (Table 3).

Table-3: Average pod setting in bagged plant of male sterile lines

CMS Lines	Pod setting
ICPA 2043(A_1)	6
ICPA 2078(A_2)	8
ICPA 2047-24(A_3)	2
ICPA 2092(A_4)	0
ICPA 2051(A_5)	2

The maximum pollen sterility was observed for the line ICPA 2078 (99.72 percent) having C.V. (0.38) followed by ICPA 2047-24 (99.58 percent) having C.V. (0.38), ICPA 2043 (99.30 percent) C.V. value (1.22)

and ICPA 2051 (97.14 percent) whereas lowest pollen sterility was found for ICPA 2092(96.90per cent) had C.V. (2.51) (Table 4). Although all male sterile lines were found suitable for Ranchi condition. The pollen fertility of maintainer lines were observed maximum for ICPB 2047-24 (B_3) i.e. 96.26 percent followed by ICPB 2092 (93.96 percent), ICPB 2078(93.79 percent), ICPB 2051(91.39 percent) and lowest 90.56per cent for ICPB 2043 (B_1). Although all lines were found up to the mark as per standard pollen fertility percentage required for B lines (Table 5).

Table: 4 Pollen Sterility (percent) of male sterile lines

Varieties/Performance	Mean	S.D.	C.V.
ICPA 2043	99.30	1.21	1.22
ICPA 2078	99.72	0.38	0.38
ICPA 2047-24	99.58	0.38	0.38
ICPA 2092	96.90	2.43	2.51
ICPA 2051	97.14	1.76	1.81

Table: 5 Pollen fertility (percent) of maintainer lines

Varieties/Performance	Mean	S.D.	C.V.
ICPB 2043	90.56	2.51	2.78
ICPB 2078	93.79	2.22	2.37
ICPB2047-24	96.26	1.24	1.28
ICPB 2092	93.96	2.97	3.17
ICPB 2051	91.39	7.04	7.71

Pigeonpea is an important crop of rain-fed agriculture in tropical and sub tropical areas. It is a unique among legumes as its floral morphology allows both as well as insect aided crops pollination and it varies from place to place. Out-crossing may ranged upto 70 percent (Saxena *et al.*, 1990). The discovery of genetic male sterility coupled with the natural out-crossing opened the possibility of commercial utilization of heterosis in pigeon pea (Reddy *et al.* 1978 & Saxena *et al*. 1983). Later on cytoplasmic genetic male sterility (CGMS) came into picture and found better over genetic male sterility (GMS) as far as hybrid development is concerned. Of course, hybrid technology has successfully been used to increase the yield. A new hybrid pigeonpea technology is capable of substantially increasing the productivity and thus offering the hope of pulse revolution in the country. (Saxena & Nadrajan, 2010). Few pigeonpea hybrids developed by ICRISAT out of which very few found suitable for Jharkhand state. For development of hybrid, evaluation of male sterile line, its maintainer as well as restorer line are required to be tested at specific location. Stable male sterile line and restorer line are must for development of hybrids.

In present investigation, an attempt has been made to evaluate male sterile line (A) and its maintenance line (B) for fisher's t-value test (average performance test) and paired t-value test (difference in their means) mean performance of morphological characters and pollen sterility and pollen fertility test for A & B line, correlation coefficient for different character of A & B line to contribute towards the searching of suitable male sterile line and its maintainers A total of five male sterile lines and five maintainer lines were evaluated for Ranchi condition in paired t-test design per plant basis at GVT pulse farm for character days for 1st flowering, days to 50 percent flowering, number of primary branches, number of secondary branches, days to maturity, pod per plant and plant height.

The average performance of ICPA 2047-24 and ICPB 2047-24 were observed different for the character, days to 1st flowering, number of primary branches, number of secondary branches, number of pods per plant and same for the character days to 50 per cent flowering, plant height, days to maturity. ICPA 2092 and ICPB 2092 were found different for the character mainly days to 1st flowering and days to 50 percent flowering. They were also observed similar for rest of the characters. Mean performance of ICPA 2051 and ICPB 2051 were found significant for the characters i.e., days to 1st flowering, days to 50 per cent flowering, & number of pods per plant and non significant for the characters number of primary branches, number of secondary branches, plant height, days to maturity. For the character, number of pods per plant the similar trend was reported by many earlier workers Phad *et al*. (2005), Vaniarajan (2007) and Sreelakshmi *et al.* (2010). Average performance of the character i.e., number of primary branches was also reported by Patel *et al.*(2009). The paired t test is used to know the differences in their means of male sterile lines (A) and its maintainer lines (B). In present study, significant mean difference was observed for the several characters. For the character *viz.* number of secondary branches and number of pods per plant without bag between ICPA 2043 and ICPB 2043, ICPA 2078 and ICPB 2078 were found highly significant mean differences and on the other hand they were found non-significant mean difference for the character *viz.,* days to 1st flowering, days to 50 percent flowering, number of

primary branches, plant height, and days to maturity. Between ICPA 2092 and ICPB 2092, highly significant mean difference was observed for the character i.e., days to 1st flowering, days to 50 per cent flowering, number of secondary branches and number of pods per plant without bag.

In present experiment, the purpose of the pod setting (average) was to know the stability (purity) of the male sterile lines under field condition the zero pod setting was found in male sterile line ICPA 2092 followed by ICPA 2047-24 and ICPA 2051. (Both have two pod settings). Highest pod setting (8 pods) was observed for ICPA 2078 (A_2). Saxena *et al.* (1976) was reported pod setting in individual plats was 25-86 percent. The extent of pod setting was observed in CMS plants is comparable to the hybridization among fertile pigeonpea cultivars. Although all male sterile line performed well under Ranchi condition. Line ICPA 2092 was found best followed by ICPA 2047-24 (A_3) and ICPA 2051 (A_5).The survival percentage was studied to know the best survived male sterile and maintainer line. The highest survival percentage was observed for ICPA 2051 (A_5) followed by ICPA 2047-24 (A_3), whereas among maintainer lines, highest survival percentage was observed for ICPB 2051 (B_5) followed by ICPB 2047-24 (B_3). On laboratory condition, the pollen sterility percentage in sterile line were observed highest for ICPA 2047-24 (A_3) followed by ICPA 2043 (A_1), whereas ICPA 2051 (A_5) had 97.14 percent of pollen sterility. Among maintainer lines, highest pollen fertility percentage was observed in ICPB 2047-24 line followed by ICPB 2092. Lowest pollen fertility was observed in line ICPB 2043 followed by ICPB 2051.

Similar result i.e., recommended levels of male sterility (>95 per cent) was also recorded by Saxena *et al.*, 2005. Saxena, 2008, Saxena *et al*., 2010a also recorded pollen sterility of 100 and 99 per cent in CMS lines in pigeonpea which tends enough support to present findings. Gangawar & Bajpai, 2005 and Dheva *et al.* 2008b also supported their findings.

CONCLUSION

Fisher t- test was computed to know about the average performance and paired t-test was calculated to study the mean difference in pairs of CMS lines and its maintainers for the different quantitative characters. On the basis of average pod setting in bagged plant of male sterile lines, the pod setting value is very negligible. The highest was found for ICPA 2078 (8 pods) and lowest for ICPA (zero pod). The result obtained in the present investigation indicated that all the A lines were found better but ICPA 2047-24 (A_3) was found best on the basis of observation of field as well as lab test. None of the lines were found significantly different for all morphological characters. In future, for development of hybrid line at Ranchi location male sterile ICPA 2047-24 (A_3) may be considered for hybrid seed production.

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