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EFFECT OF TEMPERATURE ON STERILITY IN CMS LINES OF PIGEONPEA

[*Cajanus cajan*(L.) Millsp.]

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KEYWORDS

CMS

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ICPA

Sterility

Maintainer and Restorer

ABSTRACT

Present study includes five CMS lines of Pigeonpea with A4 cytoplasm at two locations viz. ICRISAT Patancheru (Telangana) and BAU Sabour (Bihar). Observations on each plant for pollen sterility were recorded at the onset of flowering in September. The second round of sterility evaluation was done in December. The final observations were recorded in February. Result of study revealed that higher temperature of ICRISAT Patancheru (Telangana) have positive effect on the sterility level and higher sterility was reported in all CMS lines(> 90%) whereas at BAU Sabour temperature respectively low so sterility level was also low (< 90%).

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1 Introduction

Recently cytoplasmic male sterility (CMS) technology has emerged as a promising approach for dramatic enhancement of Pigeonpea (*Cajanus cajan*) productivity (Saxena, et al., 2015; Bohra et al., 2016). Hybrids pigeonpea had significantly higher seed yield over check in pigeonpea variety Asha and Maruti (Kumar et al., 2017). For yield in CGMS based hybrid pigeonpea, the standard heterosis over Maruti was up to 47.55% (Kumar et al., 2016). CMS-based hybrid breeding requires three components: sterile (A), maintainer (B) and restorer (R) lines. The B line helps in maintaining of sterility in A-lines and the R-line contains a nuclear R_f gene that restores fertility in “A × R” progenies (Chen & Liu, 2014; Kumar et al., 2016).

Cytoplasmic-nuclear male-sterility (CMS) systems have played an important role in exploiting hybrid vigor for enhancing productivity in field crops as well as in horticultural crops (Saxena, 2005). Perhaps pigeonpea is the first food legume where commercial hybrids are being bred (Saxena, 2006). Stable CMS lines play a pivotal role in pure hybrid seed production. Presently, pigeonpea have several stable CMS lines (Saxena & Kumar, 2003; Mallikarjuna & Saxena, 2005; Saxena, et al., 2005). It is found that, a highly stable male sterile line across environments and years require for pure hybrid seed production in pigeonpea (Dalvi et al., 2008; Saxena et al., 2010). The CMS line ICPA 2039 has been used to develop other CMS lines, with various desirable traits and maturity duration and with wide adaption to diverse environments of India (Saxena, 2008).

Current speculation about global climate change is that most agricultural regions will experience more extreme environmental fluctuations (Solomon et al., 2007). Temperature plays vital role on CMS lines. The objective of this study was to evaluate cytoplasmic male sterile lines for stability across both the location and effect of temperature on reference set of lines.

2 Materials and methods

The experimental materials in present study include five CGMS lines (ICPA 2043, ICPA 2092, ICPA 2078, ICPA 2048 and ICPA 2047) of pigeonpea with A_4 cytoplasm. These were obtained from International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) Patancheru, where they had been developed by manual hand pollination under insect-proof selfing net. The CMS system derived from *C. cajanifolius* (accession ICPW 29) was designated as A_4 (Saxena et al. 2005), and it is an excellent male-sterility system because of its high stability across environments (Sawargaonkar, 2011). The F_1 hybrid plants derived from this CMS produce excellent pollen load and pod set. At present, the A_4 CMS system is being used by pigeonpea breeders in India,

Myanmar and China for genetic diversification of A-lines and to produce commercial hybrids.

Experimental material was evaluated during *khariif* 2012-13 at two distinct locations, one at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Telangana and another at Bihar Agricultural University (BAU) Sabour, Bihar. ICRISAT lies at an altitude of 545 m above sea level, latitude of 17° 53' N and longitude of 78° 27' E. The soil of the experimental site was black and classified as Vertisols. The location of BAU falls in the Middle Gangetic plain region of Agro-climatic Zone III A. It is situated between 25°50' N latitude and 87°19' E longitude at an altitude of 52.73 meters above mean sea-level.

Sowing of experimental materials at ICRISAT and BAU was done in *Khariif* 2012 under insect-proof nylon net with pore size of 0.5 mm to protect the experimental materials from pollinating insects. The cytoplasmic male sterile lines were evaluated in two-row plots of 4 m length with inter and intra row spacing of 75 and 30 cm respectively. Border rows were planted to increase the precision of study and reduce border effect. All the agronomic practices were followed in these CMS lines as per to keep the crop in good condition.

Observations were recorded on each plant for pollen sterility at the onset of flowering in September. The second round of sterility evaluation was done in December, when the plants face low temperature. The final observations were recorded in February, when the temperatures rose again.

To identify fertility/sterility of pollen grains, 2% acetocarmine solution was used. Five well developed flower buds were collected randomly from different parts of each plant at the time of anthesis (9-10 AM). From each bud, the anthers were collected on a glass slide and crushed with a drop of 2% acetocarmine stain and examined under a light microscope. The count of sterile pollen grains in 10X microscopic fields was noted, five such microscopic fields were examined under each slide. The round and well stained pollen grains were counted as fertile while shriveled and unstained pollen grains were scored as sterile. The means for all the microscopic fields were worked-out and the proportion of fertile and sterile pollens was expressed in percentage on total in individual plants. The mean value of pollen sterility of all plants was considered as pollen sterility (%) for that genotype. Based on the number of stained and unstained pollen grains, the sterility status of CMS lines were computed as follows:

Pollen sterility(%)

$$= \frac{\text{No. of sterile (unstained, shriveled hyaline) pollens}}{\text{Total number of pollen grains examined}} \times 100$$

2. Results and Discussion

Results of study presented in table 1 showed significant difference for pollen sterility level in month of September, December and February at BAU Sabour, but at ICRISAT only significant difference in month of September. These results indicated highly significant differences at BAU but not at ICRISAT. All the CMS lines were analyzed for pollen sterility (%) at both of locations. The mean performance of CMS lines for pollen sterility is given in Table 2.

At ICRISAT, pollen sterility (%) ranged from 98.80 to 95.65 % in September, 94.40 to 92.10 % in December and 98 to 95% in month of February. The highest pollen sterility was recorded in ICPA 2092(98.80%) and this was followed by ICPA 2043(98.50%), ICPA 2048(96.60 %), ICPA 2078(95.845%) and ICPA 2047(96.65%) in September. While in case of December, highest CMS was reported in ICPA 2048(94.40 %) and this followed by ICPA 2043(93.40 %), ICPA 2092 (92.90 %), ICPA

2047 (92.10%) and ICPA 2078(92.10 %). In month of February, maximum CMS was reported in ICPA 2043 (98%) and this was followed by ICPA2092 (97%), ICPA 2048 (95.90%), ICPA 2047 (95.60%) and ICPA 2078 (95%) (Table 2). At ICRISAT temperature variation was low (Table 3) and because of this all the five CMS lines have good sterility level (>92%) throughout the study period and these results are agreement with finding of Makelo et al. (2013). Overall performance of all CMS lines was good at ICRISAT Patancheru for hybrid seed production.

At BAU Sabour, in month of September, sterility was ranges from 98.70 to 94.40 %, maximum in ICPA 2092 (98.70%) and this was followed by CMS of ICPA 2043 (98%), ICPA 2048 (96.40%), ICPA 2047 (94.90%) and ICPA 2078 (94.40%). Further, in the month of September average temperature varies from 24.45 to 30.80°C (Source: department of metrology BAU Sabour) so performance of CMS lines was initially good. From September temperature was continuously going down (Table 3). In month of December, average temperature was very low (15.04°C)

Table 1 Analysis of variance

		ICRISAT			BAU		
		Pollen Sterility					
SV	DF	September	December	February	September	December	February
Treatments	4	4.39**	1.87	2.86	7.03**	19.99**	24.75**
Replication	1	2.30	12.1	8.1	0.4	0.576	0.14
Error	4	0.275	2.37	0.66	0.25	2.21	0.89

Table 2 *Per se* performance of CMS lines for pollen sterility (%) at both the locations

		Pollen Sterility %							
		ICRISAT Patancheru				BAU SABOUR			
CGMS Lines	SEP	DEC	FEB	Average	SEP	DEC	FEB	Average	
ICPA 2043	98.51	93.40	98.00	96.64	98.00	71.60	83.50	84.37	
ICPA 2047	95.65	92.10	95.60	94.45	94.90	69.40	80.50	81.60	
ICPA 2048	96.60	94.40	95.90	95.63	96.40	74.30	85.00	85.23	
ICPA 2078	95.85	92.10	95.00	94.32	94.40	70.90	80.00	81.77	
ICPA2092	98.80	92.90	97.00	96.23	98.70	77.40	88.60	88.23	
Mean	97.08	92.98	96.30	95.45	96.48	72.72	83.52	84.24	
Max.	98.80	94.40	98.00	96.64	98.70	77.40	88.60	88.23	
Min.	95.65	92.10	95.00	94.32	94.40	69.40	80.00	81.60	
SEm ±	0.37	1.09	0.57	-	0.35	1.05	0.67	-	
CV (%)	0.54	1.66	0.84	-	0.52	2.04	1.13	-	

Table 3 Ranges of temperature at both the location during crop season (Source: Department of Metrology, ICRISAT& BAU)

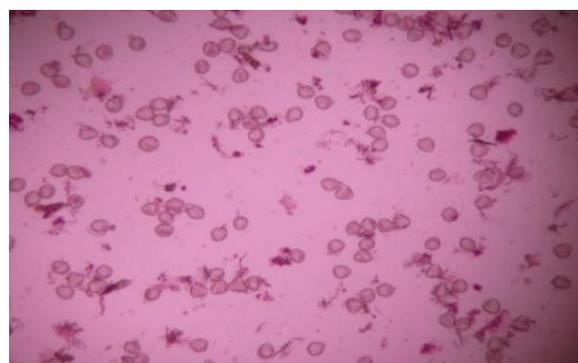
Year	Month	ICRISAT			BAU SABOUR		
		Max Temp	Min Temp	Avrg Temp	Max Temp	Min Temp	Avrg Temp
		(in °C)	(in °C)	(in °C)	(in °C)	(in °C)	(in °C)
2012	July	30.15	22.04	26.10	32.03	24.97	28.50
2012	August	29.69	21.82	25.76	31.48	25.52	28.50
2012	September	29.77	21.67	25.72	30.80	24.45	27.63
2012	October	30.4	17.99	24.20	31.08	20.58	25.83
2012	November	28.72	15.79	22.26	27.32	12.34	19.83
2012	December	29.85	13.67	21.76	21.38	8.70	15.04
2013	January	30.6	15.38	22.99	20.15	5.80	12.98
2013	February	31.06	16.34	23.70	24.73	9.03	16.88

and sterility varies from 77.40 to 69.40% (Table 2). Maximum sterility was reported in ICPA2092 (77.4 %) and this was followed by ICPA 2048 (74.30 %), ICPA 2043 (71.60%), ICPA 2078 (70.90%) and ICPA 2047 (69.40%). From December to January crop was highly damage by frost that's lead to heavy flower drop and specially in January, when plant was facing lowest temperature (20.15 to 5.80 °C), that time new flower are not occurs. In the month of February, again temperature was raising (Table 3) and simultaneously sterility level in CMS lines was also increased. In February, maximum sterility was reported from the genotype ICPA2092 (88.60 %) then ICPA 2048 (85%), ICPA 2043 (83.50%), ICPA 2047 (80.50%) and ICPA 2078 (80%)(Table 2). Similar result was reported by Saxena (2014) in pigeonpea. Further, Yuan (1990) also reported that higher temperature (> 30 °C) increased the sterility level while lower temperature (< 23 °C) results in fertility in CMS lines of rice. At BAU Sabour temperature variation was very high, so CMS lines have also variable sterility level throughout the study period. The

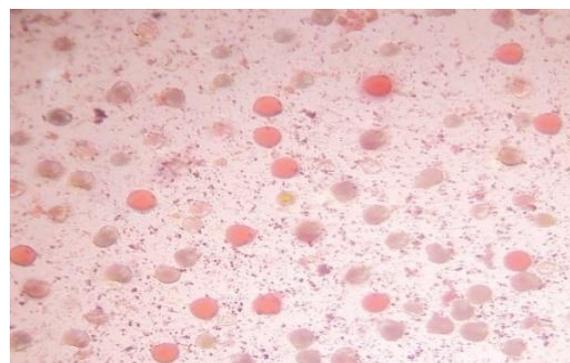
microscopic pictures are taken under light microscope clearly indicating the variation about male sterility level in CMS line with temperature (Figure 1).

In the present study, at ICRISAT highest average over season pollen sterility was recorded in ICPA 2043(96.64 %) which was followed by ICPA 2092 (96.23 %), ICPA 2048 (95.63 %), ICPA 2047(94.45 %) and ICPA 2078 (94.32 %). All the CMS lines performed well with high (>94 %) pollen sterility. Similar results were earlier reported by Dalvi (2007), Sawargaonkar et al. (2012) and Saxena et al. (2005) in CMS lines of pigeonpea. Makelo et al. (2013) also reported six CMS lines (ICPA 2043, ICPA 2039, ICPA 2091, ICPA 2050, ICPA 2042 and ICPA 2101), with high level of cytoplasmic male sterility. At BAU Sabour, the average sterility level were low (< 90%), so for this location these CMS lines will be not suitable for hybrid seed production (Table 2).

The result of present study indicates that, there are large effects of temperature on pollen fertility/sterility in CMS lines of pigeonpea.



Male sterility >90%



Male sterility <90%

Figure 1 Microscopic image of pollen grains of male sterile line

The sterility of CMS lines good at higher temperature (>23) and as the temperature going down sterility of CMS lines also going down, due to this, chance of self-seed production. Saxena, (2014) reported that at high temperature plants were completely male-sterile. Also in these studies, as temperatures going down, the male-sterile plants turned fertile and produced self-pollinated pods. In the next cycle of flowering as temperature rising, the same plants again transformed in to good sterility level.

Environmental elements are known to influence the expression of nuclear and cytoplasmic male sterility in some crops. Sterility and fertility changes depend on temperature variations (Janska & Mackenzie, 1993). Ariyanayagam et al. (1995), established that low temperatures induce male fertility, while high temperatures increase male sterility in sensitive pigeonpea genotypes. Therefore, in present investigation it can be conformed that the prevailing temperatures during these months played acritical role in the expressions of male-sterility/fertility of these reference lines. The male-sterility gene was found tightly linked to temperature-sensitive nuclear gene (Zhang et al., 1991; Siddiq et al., 1995). This situation occurs when microsporogenesis is aborted at pre-meiotic stage (Dundas et al., 1982). According to Kaul (1988), the premeiotic stage is highly sensitive to thermal changes because during this stage DNA synthesis takes place. These researchers are also agreement with our finding at cellular and molecular levels.

Conclusions

The present study was aimed to identify the suitable male sterile lines for use in the hybrid pigeonpea breeding programmes at both the locations. For proper exploitation and commercial use of CMS lines, it requires highly stable male sterile line, to ensure genetically pure F₁ hybrid seed. All the CMS lines were highly stable at ICRISAT but not at BAU Sabour. This is basically due to low temperature at BAU Sabour. For hybrid seed production at those environments that facing low temperature must be have stable CMS line at low temperature. There is need to identify sources of tolerance/ resistance CMS lines for hybrid Pigeonpea seed production in Bihar.

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Conflict of Interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise

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