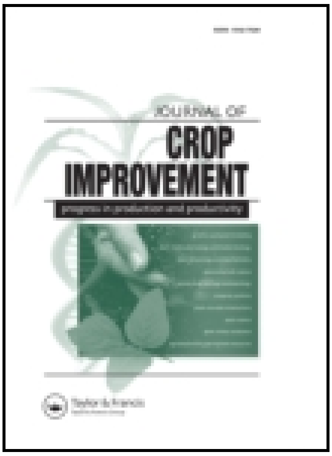

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Stability of Cytoplasmic Genetic Male Sterility and Fertility Restoration in Pigeonpea

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*In cytoplasmic genetic male sterility-based (CGMS) hybrid seed production, instability of expression of male-sterility and fertility restoration across a wide range of environments are two of the major difficulties. Therefore, the present study was carried out to investigate the stability of male sterility of nine CGMS lines under three dates of sowing and the fertility restoration of 10 CGMS-based pigeonpea (*Cajanus cajan *(L.) Millsp.) hybrids at three different locations. Significant variability existed for pollen fertility among hybrids and sterility among cytoplasmic male sterile (CMS) lines. All the hybrids except ICPH 3494 and ICPH 3491 exhibited high (>80%) pollen fertility across locations. Hybrids ICPH 2671, ICPH 2740, and ICPH 3933 had 100% male-fertile plants across locations. All the CMS lines had completely male-sterile plants across sowing dates. The CMS lines BRG1 A, Hy3C A, BRG3 A, and TTB7 A exhibited 100% pollen sterility at different sowing dates. The pooled analysis revealed a significant genotype × environment interaction for pollen fertility and sterility. The genotypic main effect + GE (GGE) biplot of hybrids showed that hybrids ICPH 2671, 2740, 3933, and 3461 were stable for fertility restoration. With the exception of ICPA 2047 and ICPA 2051, all the CMS lines were highly stable with high mean performance and least distance from AEA (average environmental axis). Male-sterility in A_4 cytoplasm*

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was independent of environmental conditions. Different dates of sowing did not affect expression of male sterility of these CMS lines.

KEYWORDS male sterility, male fertility, G × E Interaction, principal component analysis, GGE Biplot

INTRODUCTION

Pigeonpea (*Cajanus cajan* (L). Millsp.) is an important pulse crop of rainfed agriculture in the tropical and subtropical areas. It is cultivated worldwide on 5.83 million hectares with an annual production of 4.40 million tons and average productivity of 754.9 kg ha^{-1} (FAOStat 2012). Cytoplasmic-genetic male sterility (CGMS) has been used for a long time to increase the productivity of different cereals crops through hybrid development. The discovery of stable CMS system (Saxena et al. 2005) and breeding of commercial hybrids in pigeonpea are a landmark achievement. This new hybrid pigeonpea-breeding technology is capable of substantially increasing the productivity of pigeonpea, and becoming a trigger for pulse revolution in the country (Saxena and Nadarajan 2010). The development of the world's first CMS-based commercial hybrid ICPH 2671 in pigeonpea provides an opportunity of achieving the long-cherished goal of breaking the yield barrier in this crop (Saxena et al. 2013). Saxena et al. (2011) reported the genetics of fertility restoration and revealed that fertility restoration in A_4 CMS system of pigeonpea is controlled by either one or two fertility-restoring genes. They also reported that hybrids with a single fertility-restoring (*Fr*) gene produced less quantity of pollen grains than those with two *Fr* genes. This phenomenon also affects the stability of fertility restoration in hybrids across environments. The hybrids with one *Fr* gene were unstable and the extent of male-fertility varied across locations. On the contrary, the hybrids with two *Fr* genes showed a high level of fertility restoration in diverse environments. For the successful production of high-yielding CMS-based hybrids, the expression of male sterility and fertility restoration should be stable across diverse environmental conditions. Therefore, the present investigation was conducted to study the stability of male sterility and fertility restoration under different environmental conditions.

MATERIALS AND METHODS

The experimental materials consisting of 10 hybrids and two standard check varieties obtained from ICRISAT, were evaluated at Patancheru, Andhra Pradesh, India (17°53'N, 78°27'E, 545.0 MSL), Birsa Agriculture University, Ranchi, Jharkhand (23°17'N, 85°19'E, 625.0 MSL), and College of Agriculture, Sehore, Madhya Pradesh, India, (23°12'N, 77°05'E, 498.8 MSL) during 2012-13. Six-row plots were planted in 4 m-long rows with inter and intra-row spacing of 75 cm and 50 cm respectively. Simultaneously, nine CMS lines derived from *Cajanus cajanifolius* (A_4) cytoplasm were also planted at Patancheru on August 7, September 11, and October 18, 2012, to study their stability for the expression of male sterility under varying environmental conditions. Observations were recorded on all the plants at the initial flowering stage on pollen fertility (%), number of fully male fertile plants (%), partially male fertile plants (%), partially male sterile plants (%) and completely male sterile plants (%) (Khin Lay 2011). Every plant of every hybrid and CMS line was tested for pollen fertility/sterility status. To identify fertility/sterility of pollen grains, 2% aceto-carmine solution was used. Five well-developed flower buds were collected randomly from different branches of the plant at the time of anthesis (9–10 a.m.). From each bud, anthers were crushed on a glass slide and stained with a drop of 2% aceto-carmine and examined under a light microscope. The number of sterile and fertile pollen grains was recorded using a 10x magnification under light microscope. Five microscopic fields were examined on each slide. The round and well-stained pollen grains were regarded as fertile, whereas shriveled, hyaline, and unstained pollen grains were scored as sterile. The means for all the microscopic fields were calculated and the proportion of fertile and sterile pollen grains was expressed in percentage for individual plants. According to pollen-fertility status, plants were classified into four groups. The plants showing >80% pollen fertility were considered fully male-fertile and those with 40%–80%, 10%–39%, and <10% pollen fertility were respectively considered as partially male fertile, partially male sterile, and completely male sterile plants. Pooled analysis of variance (ANOVA) was carried out using the mixed procedure of the SAS software version 9.3 for Windows (SAS Institute Inc. 2011, Cary, NC). Stability of fertility restoration in hybrids and expression of male sterility in CMS lines were determined using genotype (G) + genotype × environment (GE) (GGE) biplot analysis (Yan et al. 2000) that compares a set of genotypes with a reference 'ideal' genotype, which has the highest average value of all genotypes and is absolutely stable. The percent data were transformed before analysis using square-root transformation.

RESULTS AND DISCUSSION

The genotypic differences among hybrids were highly significant ($P < 0.01$) for pollen fertility (%), number of fully male fertile plants (%), partially male fertile plants (%), partially male sterile plants (%), and completely male sterile plants (%) at all three locations, indicating the presence of substantial variation among hybrids for fertility restoration (ANOVA not presented). The mean genotypic values from different locations were subjected to pooled analysis that revealed significant genotypic differences for all the traits (Table 1). The mean square attributable to G × E interaction was highly significant for all traits except partial male-sterility (Table 1). The stability analysis by GGE biplot was performed for pollen fertility (%) and fully male fertile plants (%).

Mean Performance of Hybrids

The pollen fertility is an important character to assess fertility restoration that assures seed-set in hybrids. The mean performance of hybrids for pollen fertility and other characters at individual locations and that pooled across environments are given in Table 2. The extent of pollen fertility among hybrids ranged from 58.5% to 98.3% across locations. High pollen fertility indicated higher fertility restoration and vice versa. Among hybrids, the highest pollen fertility was recorded in ICPH 2740 (96.5%) at Patancheru, whereas ICPH 2671 recorded the highest pollen fertility (96.2% and 95.9%) at Ranchi and Sehore. All the hybrids except ICPH 3494 and ICPH 3491 exhibited high (>80%) pollen fertility and fully male- fertile plants across locations. The results are in close conformity to the findings of Wanjari et al. (2007), as they reported different hybrid combinations of pigeonpea with high (>80%) pollen fertility. The pollen fertility of a hybrid represents the restoring ability of its pollen parent when crossed to a male-sterile line. Among the hybrids tested, ICPH 2671, ICPH 2740, ICPH 3933, ICPH 2751, and ICPH 3461 had common male parent (ICPL 87119) but different female parents, and all these hybrids recorded >90% pollen fertility and 100% male fertile plants across locations, exhibiting their superior fertility restoration ability. It also indicated that the parent ICPL 87119 was the best fertility restorer and it should be utilized in further hybrid breeding programs. Similarly, Singh and Bajpai (2005), Saxena (2005), and Nadarajan et al. (2008) also reported many hybrid combinations with good fertility restoration.

Stability of Hybrids

The biplot analysis is shown in Figures 1 and 2. The results of principal component analysis (PCA) of genotype × environment interaction (GEI) showed that the first two principal components in the biplot explained 99.9% of the total variation in GEI in both the figures (Cooper and Delacy 1994). The ranking of 10 pigeonpea hybrids and two standard check cultivars based on their mean pollen fertility and fully fertile male plants with their stability performance is shown in Figure 1 and Figure 2. The genotype location closer to AEC (average environment coordinate) indicates higher mean performance. The line that passes through the origin and is perpendicular to the average environment axis (AEA) represents the stability of genotypes. Distance in

TABLE 1 Analysis of variance (F values) across the different locations and dates of sowing for hybrids and CMS lines respectively

	Hybrids						CMS lines		
Source of variation	df	Pollen fertility (%)	Fully male-fertile plants (%)	Partially male-fertile plants (%)	Partially male-sterile plants (%)	Completely male-sterile plants (%)	df	Pollen sterility (%)	Completely male-sterile plants (%)
Replication (Location)	3	1.72	2.65	2.36	1.75	0.58	3	2.63	1.12
Genotypes	11	145.61**	279.54**	74.75**	30.24**	53.76**	8	25.20**	19.57**
Locations	2	12.16**	47.13**	15.74**	1.12	18.59**	2	20.11**	2.42
Genotypes × Locations	22	2.87*	14.67**	5.61**	0.56	6.34**	16	2.66*	1.55
Residual estimate	33	0.002	0.003	0.141	0.325	0.135	24	0.002	0.003

*, ** Significant at 0.05 and 0.01 probability level, respectively.

TABLE 2 Mean performance of hybrids for pollen fertility and other characters at three different locations

S. No	Name of Hybrids	Pollen fertility (%)				Fully male-fertile plants (%)				Partially male-fertile plants (%)				Partially male-sterile plants (%)				Completely male-sterile plants (%)			
		LI†	LII†	LIII†	Pooled	LI†	LII†	LIII†	Pooled	LI†	LII†	LIII†	Pooled	LI†	LII†	LIII†	Pooled	LI†	LII†	LIII†	Pooled
1	ICPH 2671	93.4	96.2	95.9	95.3	100.0	100.0	100.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	ICPH 2740	96.5	95.7	95.4	95.9	100.0	100.0	100.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	ICPH 3933	93.6	90.0	89.8	91.2	100.0	100.0	100.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	ICPH 2751	86.4	94.3	92.6	91.4	89.5	100.0	100.0	98.7	8.5	0.0	0.0	1.7	1.6	0.0	0.0	0.4	0.0	0.0	0.0	0.0
5	ICPH 3477	83.0	92.9	90.7	89.2	85.5	100.0	100.0	98.2	6.1	0.0	0.0	1.3	1.6	0.0	0.0	0.4	6.1	0.0	0.0	1.3
6	ICPH 3461	90.0	92.2	90.6	91.0	98.9	100.0	100.0	99.8	1.6	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	ICPH 3762	83.4	85.9	84.6	84.6	80.2	100.0	92.5	94.1	15.6	1.6	5.1	6.3	3.3	1.6	1.8	2.2	0.0	0.0	0.0	0.0
8	ICPH 4490	81.1	86.1	86.2	84.5	83.6	86.3	93.2	88.1	12.2	8.4	6.8	9.0	4.2	3.4	0.0	2.1	0.0	0.0	0.0	0.0
9	ICPH 3491	48.2	64.0	67.2	60.1	46.8	64.6	52.8	55.0	14.8	10.3	23.7	15.8	17.0	18.7	18.3	18.0	21.3	6.1	3.4	8.9
10	ICPH 3494	55.1	58.0	62.1	58.5	56.3	53.6	51.0	53.6	12.5	15.6	19.6	15.8	12.5	17.4	14.5	14.7	18.7	12.9	14.1	15.1
	Check																				
11	Asha	97.7	96.5	97.6	97.3	100.0	100.0	100.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
12	Maruti	98.4	98.5	98.1	98.3	100.0	100.0	100.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Mean	**86.87**	**89.76**	**89.48**	**88.74**	**93.11**	**97.87**	**97.14**	**96.29**	**4.06**	**1.59**	**2.31**	**2.58**	**2.00**	**1.67**	**1.29**	**1.65**	**1.74**	**0.72**	**0.65**	**1.00**
	SEm±	**0.03**	**0.04**	**0.03**	**0.02**	**0.05**	**0.01**	**0.02**	**0.02**	**0.30**	**0.30**	**0.15**	**0.15**	**0.47**	**0.40**	**0.32**	**0.23**	**0.14**	**0.19**	**0.38**	**0.15**
	LSD at 5%	**0.10**	**0.12**	**0.10**	-	**0.18**	**0.05**	**0.09**	-	**0.95**	**0.95**	**0.49**	-	**1.47**	**1.25**	**1.00**	-	**0.44**	**0.6**	**1.19**	-
	CV (%)	**4.14**	**5.08**	**4.26**	**4.54**	**6.90**	**1.53**	**3.01**	**4.20**	**20.31**	**29.71**	**13.25**	**21.4**	**42.17**	**38.48**	**33.96**	**38.89**	**13.42**	**24.43**	**50.22**	**29.92**

† LI, LII, and LIII represent three locations: Patancheru, Ranchi, and Sehore, respectively.

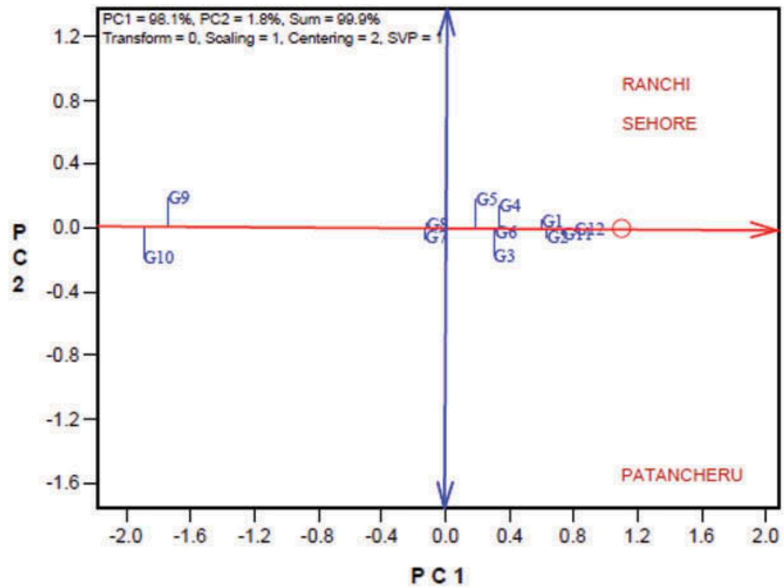

FIGURE 1 GGE biplot showing the ranking of genotypes for mean pollen fertility and stability performance over the environments.

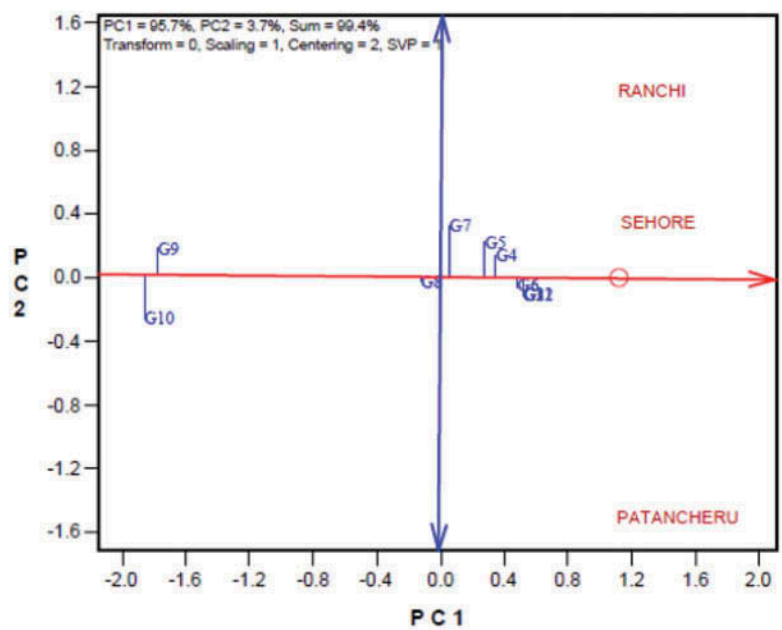

FIGURE 2 GGE biplot showing the ranking of genotypes for mean and stability performance for fully male fertile plants (%) over the locations.

either direction away from the biplot origin on this axis indicates greater G × E interaction and reduced stability. The genotypes on the right side of the perpendicular line (Figure 1 and Figure 2) had better than average performance and the genotypes on the left side of this line had lesser than mean performance. For selection, the ideal genotypes are those with both high mean pollen fertility and high stability. In the biplot, the genotypes are farthest from the origin on right side of perpendicular line and have the shortest vector length from the AEA. The genotypes Maruti (G12), Asha (G11), ICPH 2671 (G1), ICPH 3461 (G6), ICPH 2740 (G2), and ICPH 3933 (G3) were highly stable for pollen fertility (%) and fully male fertile plants (%) with high mean and shortest distance from AEA (Figure 1 and Figure 2), indicating their stability for fertility restoration. The genotypes located farthest from origin on left side of perpendicular line and greater distance from AEA were ICPH 3494 (G10) and ICPH 3491 (G9), indicating their instability for fertility restoration.

Relationship Among Test Environments

The inter-relationship among the test environments can also be evaluated from Figure 1 and Figure 2. The angle between the two environments from origin is related to the correlation coefficient between them. The cosine of the angle between two environments approximates the correlation coefficient between them (Kroonenberg 1995; Yan 2002). Acute angles (<90°) indicate a positive correlation, obtuse angles (>90°) a negative correlation, and right angles (=90°) indicate no correlation (Yan and Kang 2003). Based on the angles between location of environment on biplot, all three environments (Patancheru, Ranchi, and Sehore) were found to be positively correlated with acute angle (<90°) among them. The Sehore and Ranchi were the most discriminating environments along with a relatively small angle (<45°) with the AEA and the genotypes nearer to these two environments exhibited higher performance for pollen fertility and fully male-fertile plants at these two locations.

Stability of CMS Lines

The CMS lines BRG3 A, Hy3C A, BRG1 A, and TTB7 A had 100% pollen sterility and completely male-sterile plants indicating that these lines performed better and unable to produce fertile pollen grains at the three different dates of sowing. Among the A-lines of ICRISAT, ICPA 2039 (99.8%), ICPA 2092 (99.5%), and ICPA 2043 (99.1%) recorded highest pollen sterility across the different dates of sowing (Table 3). All the CMS lines performed well with high (>95%) pollen sterility and had completely male sterile plants at different dates of sowing (Table 3). Similar results were earlier reported by Dalvi (2007) and Makelo et al. (2013) at different locations in CMS lines

TABLE 3 Mean performance of CMS lines for pollen sterility and other characters over different dates of sowing

		Pollen sterility (%)				Completely male-sterile plants (%)				Partially male-sterile plants (%)				Partially male-fertile plants (%)				Fully male-fertile plants (%)			
S. No.	CMS lines	SI†	SII†	SIII†	Pooled	SI†	SII†	SIII†	Pooled	SI†	SII†	SIII†	Pooled	SI†	SII†	SIII†	Pooled	SI†	SII†	SIII†	Pooled
1	ICPA 2039	99.0	99.9	100.0	99.8	100.0	100.0	100.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	ICPA 2043	98.3	99.2	99.6	99.1	98.9	100.0	100.0	99.8	2.1	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	ICPA 2047	93.3	94.9	98.1	95.7	89.7	93.9	98.9	94.9	4.2	2.1	0.0	2.1	0.0	2.1	0.0	0.7	6.3	2.1	2.1	3.5
4	ICPA 2051	96.2	99.1	99.9	98.9	100.0	100.0	100.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	ICPA 2092	99.1	99.5	99.8	99.5	100.0	100.0	100.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	BRG3A	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	Hy3C A	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8	BRG1A	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
9	TTB7 A	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Mean	**99.16**	**99.65**	**99.88**	**99.62**	**99.72**	**99.88**	**99.97**	**99.88**	**0.69**	**0.23**	**0.00**	**0.31**	**0.00**	**0.23**	**0.00**	**0.08**	**0.69**	**0.23**	**0.23**	**0.39**
	LSD at 5 %	**0.10**	**0.14**	**0.07**	-	**0.15**	**0.07**	**0.15**	-	-	-	-	-	-	-	-	-	-	-	-	-
	SEm±	**0.03**	**0.04**	**0.02**	**0.02**	**0.05**	**0.02**	**0.05**	**0.02**	-	-	-	-	-	-	-	-	-	-	-	-
	CV (%)	**2.98**	**4.03**	**2.08**	**3.13**	**4.48**	**1.88**	**4.27**	**3.73**	-	-	-	-	-	-	-	-	-	-	-	-

† SI, SII, and SIII represent the first, second, and third date of sowing, respectively.

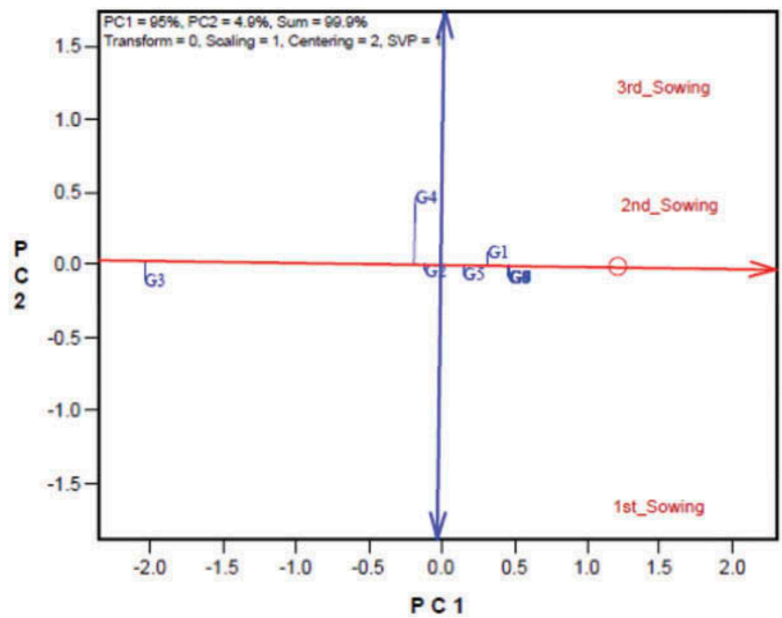

FIGURE 3 GGE biplot showing the ranking of CMS lines for mean and stability performance for pollen sterility (%) over the different dates of sowing.

of pigeonpea, while Sawargaonkar et al. (2012) and Chaudhari et al. (2014) reported these CMS line as stable for expression of male-sterility under different month temperature at ICRISAT Hyderabad. The GGE biplot for pollen sterility (%) of CMS lines (Figure 3) showed that CMS lines BRG3 A (G6), Hy3C A (G7), BRG1 A (G8), and TTB7 A (G9) were highly stable with high mean and very close to AEA. Among the CMS-lines of ICRISAT, ICPA 2039 (G1), ICPA 2092 (G5), and ICPA 2043 (G2) recorded high pollen sterility, which deviated from average mean with shorter distance from AEA indicating their stability for expression of male sterility across the different dates of sowing. All the CMS lines had highest pollen sterility when they were sown in October (3^{rd} sowing), whereas the CMS lines observed so far from first sowing (Figure 3) indicating that the expression of male sterility in CMS lines was less in first sowing as compared to second and third sowings. The non-significant G × E interaction for completely male-sterile plants (%) indicated that all CMS lines were stable for expression of male sterility at different dates of sowing.

CONCLUSION

We concluded that male sterility in A_4 cytoplasm was independent of environmental conditions, and there was no effect of different dates of sowing and environment on expression of male sterility of these CMS lines. Similarly, fertility restoration and the expression of pollen fertility under different environmental conditions were also stable, which largely depends on the genetic purity of parents.

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