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Stability analysis of male sterile-derived pearl millet (*Pennisetum glaucum*) hybrids under variable growing condition

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ABSTRACT

The identification and deployment of high-yielding pearl millet [*Pennisetum glaucum* (L.) R.Br.] hybrids adapted to various stress agro-ecologies are crucial for enhancing food and nutrition security in northern India. A study was carried out during the rainy (*kharif*) seasons of 2019 and 2020 at CCS Haryana Agricultural University, Hisar, Haryana in different growing situations (early-sown and late-sown patterns and rainfed to irrigated conditions) to investigate the genotype-environment interaction (GEI) of 59 pearl millet restorer lines, male sterile lines and their derived hybrids. Pooled analysis of variance for genotype, environment and GEI was significant for days to 50% flowering, seed yield and harvest index. These results indicate the significant differences among the genotypes, various environments and response of GEI. By combining the results of regression analysis and additive main effects and multiplicative interaction (AMMI) biplot, the genotypes HMS58 A1×EMRL-14/105, HMS54 A5×EMRL-14/127 and EMRL-15/109 exhibited high mean seed yield and high stability across environments. Among the tested environments, irrigated late-sown condition for seed yield and harvest index; and rainfed early-sown condition for days to 50% flowering had the highest discrimination ability. Hence, this study can help for the grouping of pearl millet potential hybrids and allow multi-year trials for identifying the best genotypes in the northern India.

Keywords: Genotype-environment interaction, Hybrid, Male sterility, Pearl millet, Stability

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] plays a vital role as a climate-resilient and nutritious food grain crop in semiarid and arid regions of Asia and Africa (Narasimhulu *et al.* 2023). A single pearl millet hybrid can not be expected to perform well under all the environmental conditions. A cultivar planted outside its adaptation zone would suffer yield reduction due to significant genotype-environment interactions (GEI) (Sanadya *et al.* 2024). Therefore, breeding and evaluation require a subdivision of the testing environments into relatively more homogeneous groups of locations, called mega environments. In India, pearl millet growing regimes are categorized into three mega environments: A1, A, and B zones, reflecting varying annual rainfall levels (Sankar *et al.* 2021, Satyavathi *et al.* 2021). The A1 zone consists of parts of north-western India receiving below 400 mm of annual rainfall and the A zone consists of parts of northern India receiving above 400 mm of annual rainfall whereas the B zone accounts for the area in peninsular India receiving more than above 400 mm annual rainfall (Sankar *et al.* 2021, Satyavathi *et al.* 2021). Grain yield is a complex trait that is highly influenced by different environmental conditions emphasizing the importance of understanding GEI and yield stability in breeding new varieties adapted to target environments (Shukla 1972). Joint regression analysis (JRA) (Eberhart and Russell 1966) and additive main effects and multiplicative interaction (AMMI) biplot analysis (Gauch 1988, Gauch 2013) serve as valuable tools for interpreting the relationship among genotype, environment, and GEI. Under various environmental circumstances, the AMMI model has been successfully applied to crops including soybean (Zobel *et al.* 1988), maize (Crossa 1990), wheat (Nachit *et al.* 1992), pearl millet (Shinde *et al.* 2002, Lubadde *et al.* 2016) and cassava (Aina *et al.* 2007, Adjebeng-Danquah *et al.* 2017). This study aims to deepen our understanding of GEI and identify stable, high-yielding pearl millet hybrids suited to the diverse growing conditions of northern India.

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MATERIALS AND METHODS

In the present study, 4 male sterile sources (HMS 58 A_1, HMS 53 A_4, HMS 54 A_5 and HMS 30 A_{egp}) crossed with 10 restorer lines (AC 04/13, H77/833-2-202, EMRL-14/243, EMRL-14/127, EMRL-15/109, EMRL-14/111, EMRL-14/237, EMRL-14/123 EMRL-14/103, EMRL-14/105) resulting in the development of 40 crosses during the rainy (*kharif*) season of 2019 in Line × tester (L × T) mating design at CCS Haryana Agricultural University, Hisar, Haryana. Stability analysis was performed on 59 genotypes (4 male sterile lines, 10 restorer lines, 40 derived hybrids and 5 production reference hybrids) during the rainy season of 2020. The sowing time has an important bearing on production potential of a genotype. Northern India receives less rainfall therefore, farmers have to adjust sowing times and water management to suit pearl millets genotypes in this region to increase grain productivity (Sankar *et al.* 2021). Therefore, pearl millet genotypes were evaluated across 4 diverse growing conditions as early and late-sown as well as irrigated and rainfed situations of the A zone. These environments were designated as A for irrigated early sown, B for rainfed early sown, C for irrigated late sown and D for rainfed late sown. Each genotype was sown in a single row of 4.0 m in length, with row-to-row and plant-to-plant distances of 50 cm and 12 cm, respectively. Genotypes were planted in randomized block design (RBD) with three replications.

Data collection: To ensure the normal growth, all the agronomic practices were done as per the recommendation in this zone. Data collection for the seed parent (male sterile lines), were measured from the crosses of male sterile lines with their respective maintainer lines (B lines). Data for seed yield and harvest index was measured for 5 random plants of each genotype and then averaged per plant. Days to 50% flowering were recorded on a plot basis.

Statistical data analysis: The collected data were analyzed using the software OPSTAT (Sheoran *et al.* 1998). In each environment, genotypes were grown in randomized block design (RBD) with 3 replications (Panse and Sukhatme 1985). In multi-environment trials, the best linear unbiased prediction (BLUP) model (genotypic effects were taken as random) was used to obtain mean value for the studied traits (Olivoto *et al.* 2019). Regression based models were commonly employed to identify the stable genotypes in the crop plants however, it is unable to distinguish genotype variances in a non-additive manner, such as GEI. On the other hand, biplot based models like additive main effect and multiplicative interaction (AMMI) biplot was used to gain a better understanding of genotypic stability with enhanced precision (Sanadya *et al.* 2024). Evaluation for yield potential and stability was performed based on the joint regression analysis (JRA) (Eberhart and Russell 1966) and AMMI biplot models (Gauch 2013). The AMMI2 biplot used the first interaction principal component analysis (IPCA1) score *versus* IPCA2 as described by Gauch (2013).

RESULTS AND DISCUSSION

The homogeneity of variance was assessed using Bartlett's test, which facilitated the pooling of variance for seed yield, days to 50% flowering, and harvest index across 4 different environments. The ANOVA clearly indicated the significant differences among genotypes for all the assessed traits (Table 1). Furthermore, the variance attributed to different environments and the linear effect of the environment itself exhibited significant differences for all the traits, suggesting significant variations within the tested environments. These results are in agreement with Senguttuve *et al.* (2021), who observed genotype-dependent variations in rice genotypes under variable growing conditions. The genotype-environment interaction (GEI) was further dissected into linear (b_i) and non-linear (S^2_{di}) regression components. It is noteworthy that both the linear and non-linear components of GEI were significant for seed yield, days to 50% flowering and harvest index. Gangashetty *et al.* (2023) highlighted the significant influence of environments on agronomic traits, reinforcing the significance of environmental adaptation in pearl millet breeding programmes. To explore the GEI further, an analysis of variance (ANOVA) based on AMMI model was performed to estimate the GEI for seed yield, days to 50% flowering and harvest index. The results indicated that the first interaction principal component (IPCA1) was significant for all traits investigated, highlighting its importance in explaining the observed variations. However, IPCA2 was found to be insignificant for seed yield, suggesting a lack of substantial influence in this particular trait.

Joint regression analysis: Various stability parameters, such as mean performance, regression coefficient (bi), and squared deviation from linear regression (S^2_{di}), were calculated for 59 pearl millet genotypes for quantitative traits to assess stability over environments (Table 1). For days to 50% flowering, genotypes 1, 42, 22 and 27 exhibited a unit regression and zero deviation from regression, indicating average performance across tested environments. In contrast, genotypes 10, 12, 30 and 40 had below unit regression coefficient and zero deviation from regression, suggesting average performance in unfavorable environments. Genotype 1 exhibited >1.0 regression coefficient, indicating adaptability to favourable environment. For harvest index,

Table 1 Joint regression analysis of variance for days to 50% flowering, harvest index and seed yield in pearl millet

Source of variation	Df	Days to 50% flowering	Harvest index (%)	Seed yield (g)
Genotypes	58	32.95**	42.07**	343.18**
Environment	3	93.50**	23.59**	8927.48**
GEI	174	4.80**	29.22**	104.19**
Environment + (GEI)	177	6.31**	29.12**	253.73**
Environment (linear)	1	280.60**	70.70**	26782.43**
G × E (linear)	58	3.65**	21.31**	143.77**
Pooled deviation	118	5.30**	32.61**	82.96**
Pooled error	464	2.20	1.08	1.28

GEI, Genotype × Environment interaction. *,**significant at 5% and 1% levels, respectively.

Table 2 Estimates of mean and stability parameters for 59 pearl millet parents, hybrids and production reference cultivars

Genotype	Code	Days to 50% flowering			Harvest index (%)			Seed yield (g)		
		Mean	b_i	S^2d_i	Mean	b_i	S^2d_i	Mean	b_i	S^2d_i
HMS 58 A_1 × AC 04/13	1	45.55	1.27	0.29	17.13	3.38	18.29*	37.58	0.84	220.28**
HMS 58 A_1 × H77/833-2-202	2	43.21	-0.14	1.08	16.23	3.14	3.99*	25.67	0.48	27.64**
HMS 58 A_1 × EMRL-14/243	3	46.76	0.74	5.08**	17.49	1.93	9.11**	33.50	1.56	33.24**
HMS 58 A_1 × EMRL-14/127	4	48.81	0.54	1.27	15.28	2.46	16.84**	31.17	1.91	149.02**
HMS 58 A_1 × EMRL-15/109	5	45.47	1.92	3.37**	14.42	2.79	26.52**	24.00	0.82	57.66**
HMS 58 A_1 × EMRL-14/111	6	48.00	0.59	1.42	16.92	2.04	8.02**	38.25	1.86	69.83**
HMS 58 A_1 × EMRL-14/237	7	48.63	-0.31	0.13	11.83	-0.63	2.74*	18.92	0.43*	4.99*
HMS 58 A_1 × EMRL-14/123	8	46.57	-0.82*	-0.45	14.01	1.96	14.99**	24.92	1.18	59.79**
HMS 58 A_1 × EMRL-14/103	9	50.81	1.42	7.76**	20.43	5.12	4.81**	41.75	1.97	49.97**
HMS 58 A_1 × EMRL-14/105	10	47.18	0.46	-0.06	17.13	-3.00	10.30**	30.17	0.89	11.30**
HMS 54 A_5 × AC 04/13	11	47.79	1.03	2.19*	20.50	5.81	137.62**	28.00	1.43	87.27**
HMS 54 A_5 × H77/833-2-202	12	45.42	0.66	0.06	18.69	3.62	41.98**	28.50	0.51	33.68**
HMS 54 A_5 × EMRL-14/243	13	50.06	1.13	1.16	15.77	0.34	21.75**	21.17	0.24	45.07**
HMS 54 A_5 × EMRL-14/127	14	48.46	1.68	6.45**	21.36	8.68	9.11**	33.00	1.19	27.91**
HMS 54 A_5 × EMRL-15/109	15	47.41	1.06	1.91*	14.72	-0.72	14.86**	28.83	1.17	7.80**
HMS 54 A_5 × EMRL-14/111	16	50.09	2.34	5.29**	16.92	5.41	43.75**	38.17	0.85	21.25**
HMS 54 A_5 × EMRL-14/237	17	51.40	-0.01	1.22	17.05	0.69	8.67**	46.50	1.61	22.22**
HMS 54 A_5 × EMRL-14/123	18	52.60	-0.14	-0.19	20.30	-2.62	21.74**	38.92	0.63	169.91**
HMS 54 A_5 × EMRL-14/103	19	51.40	0.84	1.76*	16.80	-6.88	49.58**	36.42	0.47	321.78**
HMS 54 A_5 × EMRL-14/105	20	49.51	2.69	11.29**	20.02	1.04	126.71**	44.58	0.94	692.16**
HMS 53 A_4 × AC 04/13	21	43.08	0.00	0.50	15.38	-3.93	15.65**	18.75	0.45	58.78**
HMS 53 A_4 × H77/833-2-202	22	44.08	1.24	0.35	13.85	1.27	40.02**	20.25	0.30	91.58**
HMS 53 A_4 × EMRL-14/243	23	45.72	0.93	2.67*	16.01	-8.50	22.01*	38.00	1.85*	9.65**
HMS 53 A_4 × EMRL-14/127	24	46.28	-1.54*	0.07	21.51	3.72	3.19**	34.17	1.59	78.37**
HMS 53 A_4 × EMRL-15/109	25	45.67	-1.12**	-0.72	17.83	1.87	16.90**	28.67	1.50	139.35**
HMS 53 A_4 × EMRL-14/111	26	47.66	1.39	3.22**	17.61	0.10	3.50*	25.33	1.17	7.30**

Contd.

Table 2 (*Concluded*)

Genotype	Code	Days to 50% flowering			Harvest index (%)			Seed yield (g)		
		Mean	b_i	S^2d_i	Mean	b_i	S^2d_i	Mean	b_i	S^2d_i
HMS 53 A_4 × EMRL-14/237	27	45.07	1.06	-0.10	18.13	0.41	2.91*	25.58	0.87	0.70
HMS 53 A_4 × EMRL-14/123	28	46.72	-0.19	0.18	14.42	0.54	12.58**	24.83	1.22	48.55**
HMS 53 A_4 × EMRL-14/103	29	49.06	0.79	-0.43	13.06	-0.30	1.64	21.83	0.60	7.27**
HMS 53 A_4 × EMRL-14/105	30	43.66	0.40	0.76	18.72	8.28	20.22**	32.67	1.52	50.55**
HMS 30A_{egp} × AC 04/13	31	48.24	1.13	0.61	13.95	0.89	-0.30	20.92	0.63	16.32**
HMS 30A_{egp} × H77/833-202	32	49.86	0.97	5.85**	18.83	1.56	5.59**	33.00	1.18	8.12**
HMS 30A_{egp} × EMRL-14/243	33	52.31	1.46	2.54*	16.48	2.32	34.25**	23.25	0.54	150.72**
HMS 30A_{egp} × EMRL-14/127	34	48.83	2.63	0.72	14.75	-0.92	12.16**	35.08	1.79	36.83**
HMS 30A_{egp} × EMRL-15/109	35	49.00	0.28	1.07	12.73	1.18	12.18**	23.08	-0.06	39.89***
HMS 30A_{egp} × EMRL-14/111	36	54.08	0.54	1.49	16.22	1.35	1.59	36.17	1.17	94.87**
HMS 30A_{egp} × EMRL-14/237	37	52.25	0.70	2.23*	19.14	1.45	11.02**	36.17	1.50	63.77**
HMS 30A_{egp} × EMRL-14/123	38	53.08	1.70	6.28**	14.13	-3.06	10.10**	18.75	0.23	47.45**
HMS 30A_{egp} × EMRL-14/103	39	53.83	1.26	2.22*	13.59	0.55	11.39**	17.92	0.61*	2.31
HMS 30A_{egp} × EMRL-14/105	40	46.17	0.50	0.49	19.23	-0.33	7.93**	30.58	1.66	171.94**
HMS 58 A_1 (A line)	41	50.17	0.95	0.94	13.86	0.92	52.38**	15.58	0.35**	0.10
HMS 54 A_5 (A line)	42	47.50	1.28	-0.36	10.62	-1.35	16.63**	13.08	0.16	21.21**
HMS 53 A_4 (A line)	43	49.00	1.15	-0.09	15.06	2.39	15.75**	27.25	1.34	141.23**
HMS 30A_{egp} (A line)	44	50.08	1.62	-0.07	13.65	4.23	52.91**	22.83	0.87	141.51**
AC0 4/13 (R line)	45	48.25	1.50	3.27**	17.17	-4.96	18.78**	20.92	0.77	26.30**
H77/833-2-202 (R line)	46	43.62	1.01	5.13**	17.25	1.60	8.86**	19.42	0.35*	3.73*
EMRL-14/243 (R line)	47	52.17	1.15	22.83**	28.22	14.12	558.48**	34.08	2.13	369.66**
EMRL-14/127 (R line)	48	48.75	1.02	8.93**	14.42	-7.49	7.69**	23.25	0.44	192.86**
EMRL-15/109 (R line)	49	47.75	2.32	15.92**	24.49	-13.67	51.46**	53.75	2.08**	0.62
EMRL-14/111 (R line)	50	48.83	2.40	27.12**	15.73	0.13	27.85**	30.83	1.07	101.46**
EMRL-14/237 (R line)	51	51.33	1.01	6.52**	14.19	3.35	26.81**	16.83	0.28	30.22**
EMRL-14/123 (R line)	52	49.67	0.87	1.77*	17.19	-0.55	23.69**	40.58	0.32	18.99**
EMRL-14/103 (R line)	53	49.83	2.13	20.97**	15.09	2.83	14.27**	25.08	1.03	92.00**
EMRL-14/105 (R line)	54	53.08	1.79	33.92**	17.83	5.89*	-0.43	22.58	0.62	5.93**
HHB 311 (C)	55	54.50	1.05	14.14**	17.89	2.21	59.38**	29.58	0.68	55.74**
HHB 67 (C)	56	48.33	2.89	9.35**	21.83	3.48	30.83**	50.75	1.72	48.07**
HHB 335 (C)	57	51.42	1.66	10.11**	25.08	4.83	61.09**	44.42	1.56	73.40**
HHB 299 (C)	58	48.33	1.06	-0.53	14.61	-2.96	8.12**	42.75	0.65	188.39**
HHB 272 (C)	59	44.00	1.05	7.39**	17.55	1.04	29.97**	43.50	1.29	117.87**
CD (P=0.05)		3.22			8.00			12.76		

bi, Regression coefficient; S^2_d, Deviation from regression; A line, Seed parent; R line, Restorer line; C, Production reference cultivars.
*, **significant at 5% and 1% levels, respectively.

genotypes 54, 31, 36 and 29 exhibited wider adaptation. Specifically, genotypes 54 and 36 exhibited adaptation in favourable environments, while genotype 31 showed wider adaptation in unfavourable environments. For seed yield, genotypes 41, 49, 27 and 39 exhibited wider adaptation. Among them, genotypes, 41 and 49 had adaptation in favourable environments, while genotype 27 and 39 showed wider adaptation in unfavourable environments. Moreover, genotype 49 exhibited wider adaptation with high seed yield, while other genotypes including hybrids showed low seed yield over production reference cultivars. Arya and Yadav (2009) identified some white type and grey type hybrids as stable for all the tested environments among 32 pearl millet hybrids. Similar results were observed by Dadarwal *et al.* (2018) who also found some hybrids exhibited higher grain yield and stability across variable irrigation management system in north-western India. Additionally, Dutta *et al.* (2021) conducted stability analysis on 42 pearl millet hybrids under 6 environments in West Africa, identified 2 hybrids as the most stable for grain yield. In the present study, according to the JRA model, no highly stable pearl millet hybrids for seed yield were identified, possibly due to the minimal utilization of non-regression components such as GEI. Hence, employing a biplot-based model like AMMI can enhance the comprehension of GEI and aid in identifying high-stability genotypes across the 4 variable growing conditions.

AMMI biplot analysis: The GEI component was further partitioned and explained by two interaction principal component axes (IPCA), namely IPCA1 and IPCA2 (Table 2). The results of the AMMI2 biplot (IPCA1 with IPCA2) for days to 50% flowering, seed yield and harvest index are presented in Fig. 1, 2 and 3, respectively. In AMMI2 biplots, if a genotype is located close to the centre of the biplot (origin), it is considered more stable than those genotypes located farther away. Similarly, a genotype located in a respective environment sector is considered specifically adapted to that environment. For days to 50% flowering, genotypes 27, 46 and 58 were stable as they were close to centre on biplot. Genotypes 16, 20, 47 and 34 had specific adapted in irrigated early sown conditions, while genotypes 49, 50, 53 and 56 were specifically adapted in rainfed early sown conditions. Genotypes 47 and 55 exhibited specific adaptation to irrigated late sown conditions, while genotypes 8, 24 and 25 were specifically adapted to rainfed late sown conditions (Fig. 1).

For seed yield, genotypes 10, 14, 15, 26, 27 and 32 were stable genotypes as they were closed to centre on the biplot. Genotypes 35, 51, 42 and 44 showed specific adaptation to irrigated early sown conditions, while genotypes 38 and 18 were specifically adapted to rainfed early sown conditions. Genotypes 47 exhibited specific adaptation to irrigated late sown conditions, whereas genotypes 20, 19 and 49 were specifically adapted to rainfed late sown conditions (Fig. 2). For harvest index, genotypes 27, 17 and 32 were identified as stable genotypes as they were closed to centre on the biplot. Genotypes 49, 20, 19 and 23 had specific adaptation

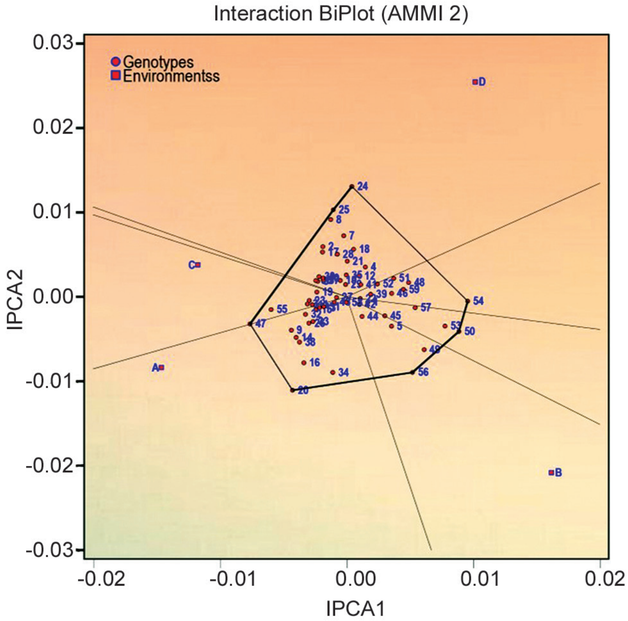

Fig. 1 The scattered distribution patterns of 59 pearl millet genotypes and 4 environments for days to 50% flowering presented in AMMI model with IPCA1 scores shown on the abscissa and IPCA2 scores shown on the ordinate.
A, Irrigated early sown; B, Rainfed early sown; C, Irrigated late sown; and D, Rainfed late sown.

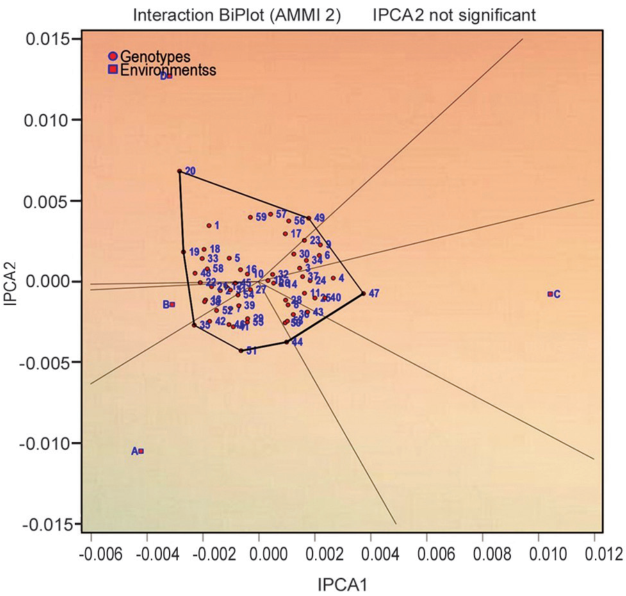

Fig. 2 The scattered distribution patterns of 59 pearl millet genotypes and 4 environments for seed yield presented in AMMI model with IPCA1 scores shown on the abscissa and IPCA2 scores shown on the ordinate.
A, Irrigated early sown; B, Rainfed early sown; C, Irrigated late sown; and D, Rainfed late sown.

to irrigated early sown conditions, while genotypes 18, 33 and 21 were specifically adapted to rainfed early sown conditions. Genotypes 47 and 11 had specific adapted to irrigated late sown conditions, while genotypes 41, 16, 22 and 14 were specifically adapted to rainfed late sown conditions (Fig. 3). Yan and Tinker (2006) highlighted that

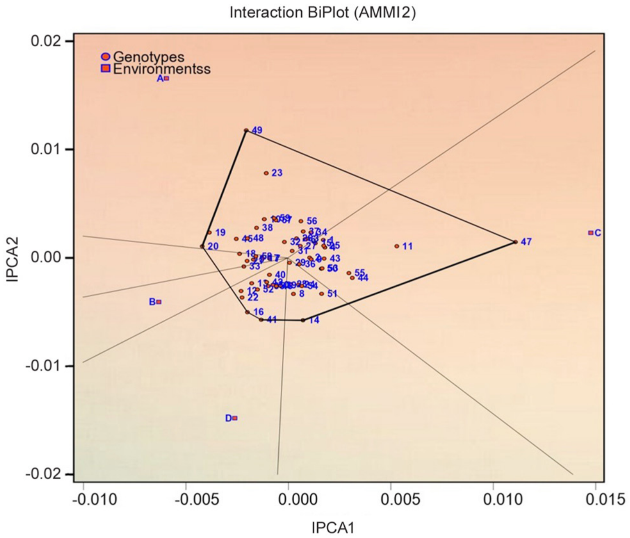

Fig. 3 The scattered distribution patterns of 59 pearl millet genotypes and 4 environments for harvest index presented in AMMI model with IPCA1 scores shown on the abscissa and IPCA2 scores shown on the ordinate.
A, Irrigated early sown; B, Rainfed early sown; C, Irrigated late sown; and D, Rainfed late sown.

better discrimination ability of the tested environments is good for selecting generally adapted genotypes. The length of environment vector form origin represents discrimination ability of environment among tested genotypes. Among the 4 tested environments, for seed yield and harvest index, irrigated late sown conditions, whereas rainfed early sown for days to 50% flowering showed the most discrimination. Pawar *et al.* (2012) found 4 hybrids that exhibited high stability in peninsular region of country. In the same region, Sumathi *et al.* (2017) evaluated 27 pearl millet hybrids for agronomic traits and identified 2 hybrids as high stability across tested environments. Asungre *et al.* (2022) investigated 24 pearl millet single-cross hybrids to estimate stability for grain yield using AMMI biplot and identified 3 hybrids had high yield with stability.

Pearl millet serves as a crucial climate-resilient staple crop in arid regions of Asia and Africa. Our study is encompassing diverse growing conditions in northern India for the identifying stable genotypes using the joint regression analysis and AMMI models. A per comprehensive analysis of JRA and AMMI models, hybrids HMS58 A1×EMRL-14/105, HMS54 A5×EMRL-14/127 and 1 restorer line EMRL-15/109 exhibited high mean seed yield with high stability. Furthermore, hybrids HMS58 A1×AC04/13, HMS58 A1×H77/833-2-202, HMS53 A4×H77/833-2-202 and HMS53 A4×EMRL-14/237 exhibited early flowering along with stability. These high seed yielding with stable genotypes can be effectively utilized in multi-location trials for potential release and hybrid seed production. Furthermore, this study holds promise for facilitating the grouping of heterotic traits, identifying stable pearl millet hybrids and the identification of novel restorer lines tailored to specific male sterile sources.

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