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ASSESSMENT OF GENETIC DIVERSITY IN PEARL MILLET FOR GRAIN YIELD, RANCIDITY AND SEED VIGOR TRAITS

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ABSTRACT

Pearl millet (*Pennisetum glaucum* L.) is an important cereal crop cultivated in arid and semi-arid regions owing to its adaptability to harsh environmental conditions and high nutritional value. However, rapid development of rancidity in flour after milling limits its storage stability and wider utilization. Therefore, identification of genotypes combining high grain yield with improved flour shelf life and seed vigour is essential for breeding programmes. The present study evaluated genetic diversity among sixty pearl millet maintainer lines grown during the kharif season at CCS Haryana Agricultural University, Hisar, in a randomized block design with two replications. Eight traits, including days to 50% flowering, plant height, panicle length, number of productive tillers, grain yield per plant, vigour index II, comprehensive acid value difference and comprehensive peroxide value difference, were analysed using hierarchical cluster analysis (Euclidean distance, Ward's method) and Mahalanobis D^2 statistics. The genotypes were grouped into five distinct clusters, indicating substantial genetic variability. Cluster mean analysis revealed significant variation for yield, rancidity and seed vigour traits. Certain clusters comprised genotypes with higher grain yield coupled with lower rancidity indices, highlighting their potential for improving flour shelf life. The results highlight substantial genetic variability and indicate that yield and rancidity-related traits are not strongly associated. The identified genotypes provide promising parental combinations for developing high-yielding pearl millet varieties with improved flour shelf life and storage stability.

Key words : Pearl millet, Genetic diversity, Cluster analysis, Rancidity, Seed vigour, Grain yield.

Introduction

Pearl millet (*Pennisetum glaucum* L.) is one of the most important cereal crops cultivated in arid and semi-arid regions of Asia and Africa due to its remarkable tolerance to drought, high temperature and poor soil fertility (Yadav *et al*., 2012; Govindaraj *et al*., 2011). The crop serves as a major source of food, fodder and nutritional security for millions of people living in marginal environments. In addition to its adaptability, pearl millet grains possess high nutritional value with appreciable levels of protein, minerals and energy compared with several other cereals (Yadav *et al*., 2012; Govindaraj *et al*., 2011; Kumar *et al*., 2021). These attributes have increased interest in pearl millet as a climate-resilient and nutritionally rich crop suitable for sustainable agriculture.

Despite these advantages, the utilization of pearl millet in processed food systems is limited by the rapid development of rancidity in flour after milling. The flour tends to develop undesirable odour and bitterness within a short period due to lipid hydrolysis and subsequent oxidation reactions (Kaced *et al*., 1984; Sharma *et al*., 2022). This deterioration reduces the shelf life of the flour and restricts its commercial potential. Therefore, identification of genotypes with improved flour stability along with good agronomic performance has become an important objective in pearl millet improvement programmes. This necessitates the identification of genotypes that combine high productivity with improved storage stability. However, simultaneous improvement of yield and storage stability remains a major challenge due to the complex interaction among these traits.

In addition to flour shelf life, seed vigour and seedling traits play a significant role in determining the establishment and productivity of pearl millet crops. Traits such as germination percentage, seedling growth and vigour indices provide valuable information regarding seed quality and the early growth potential of genotypes (Abdul-Baki and Anderson, 1973; Singh *et al.*, 2021). Integrating these traits with yield and shelf-life parameters can assist breeders in identifying promising genotypes for developing improved varieties.

Assessment of genetic diversity among breeding materials is an essential step in crop improvement programmes. Knowledge of diversity helps breeders identify genetically divergent genotypes and select suitable parents for hybridization. Multivariate techniques such as cluster analysis are widely used to classify genotypes into distinct groups based on multiple traits simultaneously (Sankar *et al.*, 2014; Patel *et al.*, 2023). Such analyses provide insights into the extent of variability present in the germplasm and facilitate the identification of genotypes possessing desirable combinations of traits. The effectiveness of such analyses largely depends on the identification of genetically diverse parents, which can be achieved through multivariate techniques such as Mahalanobis D^2 analysis.

Considering the importance of grain yield, flour shelf life and seed vigour in pearl millet improvement, the present study was undertaken to assess genetic diversity among pearl millet genotypes using agro-morphological, seed vigour and rancidity-related traits. Despite their importance, these traits have rarely been evaluated together in diversity studies, highlighting the need for an integrated approach. Previous studies have highlighted the presence of considerable genetic variability for yield and quality traits in pearl millet; however, integrated evaluation of yield, rancidity and seed vigour remains limited. Therefore, the objective of this study was to classify genotypes into distinct groups and identify promising parental lines for improving yield and storage stability in pearl millet.

Materials and Methods

The present investigation was conducted during the kharif season of 2021 at the research area of the Bajra Section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar, Haryana, India. The experimental material comprised sixty pearl millet (*Pennisetum glaucum* L.) maintainer lines representing diverse genetic backgrounds, selected to capture variability for yield, seed vigour and rancidity-related traits. The experiment was laid out in a randomized block design with two replications. Each genotype was grown in a single-row plot of 4 m length, maintaining a spacing of 45 cm between rows and 10-12 cm between plants to ensure optimum plant population. All recommended agronomic and plant protection measures were followed to raise a uniform and healthy crop.

Observations were recorded on randomly selected competitive plants from each genotype in both replications for agro-morphological traits, namely days to 50% flowering, plant height (cm), panicle length (cm), number of productive tillers per plant and grain yield per plant (g). Seed vigour was assessed through vigour index II, calculated as the product of standard germination percentage and seedling dry weight, following the method of Abdul-Baki and Anderson (1973). Germination tests were conducted under controlled laboratory conditions, and seedling dry weight was recorded after drying to constant weight. Rancidity-related parameters were estimated in terms of comprehensive acid value difference (CAV diff) and comprehensive peroxide value difference (CPV diff), calculated as the difference in respective values between the first and tenth day after milling, indicating the extent of lipid hydrolysis and oxidative deterioration (Kaced *et al.*, 1984).

Mean values across replications were used for statistical analysis. The data were standardized prior to analysis to minimize the effects of scale differences among traits. Genetic diversity among genotypes was assessed using hierarchical cluster analysis based on Euclidean distance and clustering was performed using Ward's minimum variance method. Genetic divergence was further quantified using Mahalanobis D^2 statistics to estimate intra- and inter-cluster distances. The relative contribution of different traits towards total genetic divergence was computed following the method of Singh and Chaudhary (1977). All statistical analyses were performed using standard statistical software such as INDOSTAT.

Results and Discussion

Hierarchical cluster analysis based on Euclidean distance grouped the sixty pearl millet genotypes into five distinct clusters, indicating the presence of considerable genetic variability among the experimental material (Kumar *et al.*, 2021 and Singh *et al.*, 2023). The clustering pattern revealed that genotypes were grouped based on overall similarity for agro-morphological, seed vigour and rancidity-related traits rather than their geographical origin. This level of diversity provides opportunities for selecting genetically divergent parents for hybridization and developing superior recombinants. Genetic diversity assessed through multivariate approaches has been widely utilized in pearl millet

Table 1 : Distribution of sixty pearl millet genotypes into different clusters.

Cluster	No. of genotypes	Genotypes
I	15	HMS 6B, HMS 16B, HMS 37B, HMS 41B, HMS 44B, HMS 45B, HMS 52B, HMS 55B, HMS 63B, HMS 66B, HMS 70B, HMS 73B, ICMB 94555, ICMB 04888, ICMB 843-22
II	14	HMS 7B, HMS 18B, HMS 28B, HMS 32B, HMS 34B, HMS 40B, HMS 54B, HMS 59B, HMS 60B, HMS 62B, HMS 65B, HMS 68B, ICMB 02333, ICMB 06555
III	13	HMS 13B, HMS 22B, HMS 23B, HMS 26B, HMS 33B, HMS 36B, HMS 38B, HMS 48B, HMS 49B, HMS 50B, HMS 53B, HMS 57B, HMS 58B
IV	10	HMS 14B, HMS 47B, HMS 51B, HMS 64B, HMS 69B, HMS 71B, HMS 74B, ICMB 97111, ICMB 89111, ICMB 92777
V	8	HMS 29B, HMS 30B, HMS 43B, HMS 56B, HMS 75B, ICMB 05222, ICMB 02111, ICMB 04777

Note: Distribution of genotypes into clusters based on similarity for agro-morphological, seed vigour and rancidity-related traits.

Table 2 : Intra- and inter-cluster Euclidean distances among clusters.

Cluster	I	II	III	IV	V
I	**2.41**	3.18	2.63	5.24	4.55
II	3.18	**2.76**	2.75	4.53	4.69
III	2.63	2.75	**2.12**	3.34	4.17
IV	5.24	4.53	3.34	**2.89**	4.46
V	4.55	4.69	4.17	4.46	**2.67**

Note: Values represent intra- (diagonal) and inter-cluster Euclidean distances among clusters, indicating genetic divergence.

improvement programmes to identify superior genotypes and trait combinations. Previous studies have also reported substantial variability for yield and quality traits in pearl millet, emphasizing the importance of such analyses in breeding strategies (Rajotia *et al.*, 2025). The dendrogram illustrating the clustering pattern of genotypes is presented in Fig. 1.

The distribution of genotypes across clusters was uneven, indicating differences in the degree of similarity among genotypes (Table 1). Cluster I contained the highest number of genotypes, followed by Clusters II and III, whereas Cluster V comprised the least number of genotypes. The unequal distribution of genotypes among clusters reflects the presence of varying degrees of genetic divergence among the studied material. The grouping of genotypes from diverse genetic backgrounds into the same cluster further indicates that geographical origin may not necessarily correspond with genetic divergence, which is in agreement with earlier findings in pearl millet (Upadhyaya *et al.*, 2017; Patel *et al.*, 2022).

The intra- and inter-cluster Euclidean distances among the five clusters are presented in Table 2. The maximum inter-cluster distance was observed between Cluster I and Cluster IV, indicating that genotypes belonging to these clusters are genetically most divergent and can be utilized in hybridization programmes to obtain transgressive segregants. The minimum inter-cluster distance between Cluster I and Cluster III suggested closer genetic relationship among the genotypes. Intra-cluster distances were comparatively lower than inter-cluster distances, indicating homogeneity within clusters and heterogeneity between clusters. These findings are in accordance with the principles of genetic divergence as suggested by Rao (1952) and Singh and Chaudhary (1977).

Table 3 : Cytoplasmic classification of pearl millet genotypes used in the study.

Cytoplasm	Genotypes
A1	HMS 6B, 7B, 13B, 14B, 16B, 18B, 32B, 33B, 37B, 38B, 40B, 41B, 44B, 45B, 47B, 50B, 58B, 69B, 73B, ICMB 97111, ICMB 94555, ICMB 89111, ICMB 843-22, ICMB 92777
A4	HMS 36B, 52B, 60B, 63B, 75B, ICMB 02333, ICMB 04888, ICMB 04777, ICMB 02111, ICMB 06555
A5	HMS 29B, 59B, 64B, 74B
Aegp	HMS 30B
DSA	HMS 22B, HMS 23B

Note: Cytoplasmic classification of B-lines is based on their corresponding A-line sources.

The selection of parents from clusters separated by high inter-cluster distances is expected to result in greater heterosis and wider variability in segregating generations, thereby enhancing the efficiency of breeding programmes (Singh and Chaudhary, 1977; Sharma *et al.*, 2021). In addition to genetic divergence based on quantitative traits, the cytoplasmic background of parental lines also plays

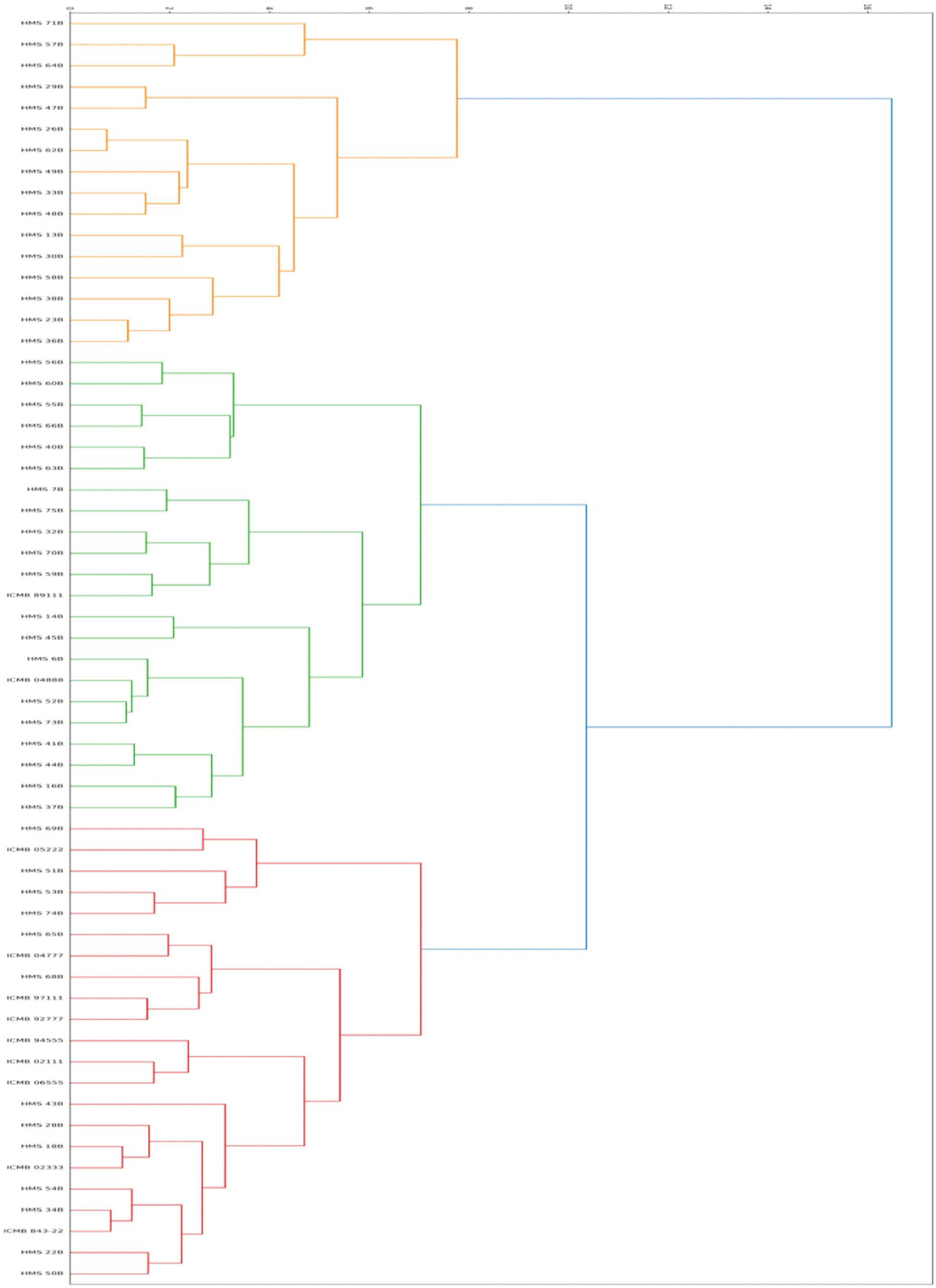

Fig. 1 : Dendrogram showing hierarchical clustering of sixty pearl millet genotypes based on Euclidean distance using Ward's method, indicating genetic divergence among genotypes.

Table 4 : Cluster mean values for agro-morphological, seed vigour and rancidity-related traits.

Cluster	Days to 50% flowering	Plant height (cm)	Panicle length (cm)	Productive tillers	Grain yield (g)	Vigour Index II	CAV diff	CPV diff
I	Mean:56.43; Range: 51.50-67.50	Mean:117.77; Range: 101.00 -139.00	Mean:18.02; Range: 14.95-20.20	Mean:3.07; Range: 1.15 -4.20	Mean:12.18; Range: 1.80 -20.00	Mean:92.87; Range: 54.60 -223.00	Mean:54.24; Range: 34.45 -113.60	Mean:75.37; Range: 44.53 -168.30
II	Mean:58.75; Range:50.50 -78.50	Mean:120.54; Range:100.50 -129.50	Mean:18.39; Range:15.62 -22.03	Mean:2.94; Range:1.95 -3.90	Mean:14.08; Range:1.30 -19.75	Mean:137.28; Range: 113.67-234.93	Mean:77.55; Range: 57.48 -127.34	Mean:108.61; Range: 85.58-181.14
III	Mean:57.73; Range: 52.00 -63.00	Mean:129.54; Range: 111.50-144.00	Mean:18.94; Range: 14.01 -22.70	Mean:3.11; Range: 1.90 -4.15	Mean:20.24; Range:2.70 -39.60	Mean:256.37; Range: 170.13-357.13	Mean: 139.63; Range:93.92 -198.37	Mean:199.60; Range:132.02 -277.75
IV	Mean:59.60; Range: 54.50-67.00	Mean:132.35; Range: 100.50-150.00	Mean:20.54; Range: 16.18-25.64	Mean:3.74; Range: 2.00-6.10	Mean:26.42; Range: 3.20-40.50	Mean:221.93; Range: 41.50-437.27	Mean:127.33; Range: 33.95 -227.93	Mean:179.94; Range: 37.73 -332.60
V	Mean:63.56; Range: 53.50-70.00	Mean:123.50; Range: 108.00-151.50	Mean:17.99; Range: 11.25-24.61	Mean:2.71; Range: 1.15-3.35	Mean:12.80; Range: 2.90-26.30	Mean:250.18; Range: 143.40-398.03	Mean:139.75; Range: 78.60-205.77	Mean: 205.77; Range:111.00 -301.90

Note: Values are cluster means for different traits, indicating variation among clusters for yield, seed vigour and rancidity-related parameters.

Table 5 : Contribution of different traits towards genetic divergence.

Trait	Times ranked first	Contribution (%)
Panicle length (PL)	318	17.97
Days to 50% flowering	302	17.06
Plant height (PH)	297	16.78
Number of productive tillers per plant (NPT)	288	16.27
Grain yield per plant (GY)	269	15.20
Vigour index II (V-II)	189	10.68
Comprehensive acid value difference (CAV diff)	93	5.25
Comprehensive peroxide value difference (CPV diff)	14	0.79

Note: Contribution (%) indicates the relative importance of each trait towards total genetic divergence among genotypes.

an important role in hybrid breeding. Cytoplasmic diversity can influence traits such as yield stability and stress tolerance. The distribution of cytoplasmic sources among the studied genotypes, based on their corresponding A-line backgrounds, is presented in Table 3. The presence of multiple cytoplasmic types indicates additional variability that can be exploited in breeding programmes for the development of superior hybrids through cytoplasm diversification.

Cluster mean values revealed considerable variation among clusters for the traits studied (Table 4). Grain yield per plant ranged from 12.18 to 26.42 g, vigour index II ranged from 92.87 to 256.37, while rancidity-related parameters such as CAV diff and CPV diff ranged from 54.24 to 139.75 and 75.37 to 205.77, respectively. This indicates distinct differences in performance for yield, seed vigour and rancidity-related traits. Cluster IV exhibited superior performance for grain yield per plant, panicle length and number of productive tillers, indicating the presence of high yielding genotypes. These results indicate that genotypes from this cluster can be effectively utilized for improving productivity in breeding programmes. However, their relatively higher rancidity values suggest the need for careful selection when targeting both yield and shelf life simultaneously. In contrast, Cluster I recorded comparatively lower values for CAV diff and CPV diff, suggesting better flour stability during storage. Cluster III showed higher values for vigour index II, indicating superior seed vigour. Therefore, genotypes belonging to these clusters may be utilized in breeding programmes for combining high yield with improved storage stability and seed quality. Similar findings have been reported by Govindaraj *et al*. (2011), Upadhyaya *et al*. (2017), Shashibhushan *et al*. (2022), Kumar *et al*. (2021) and Singh *et al*. (2023).

The comparison of cluster mean values indicates that higher grain yield is often associated with increased rancidity values, suggesting a possible physiological linkage between yield and lipid metabolism. High yielding genotypes may possess greater lipid content or metabolic activity, which could accelerate lipid hydrolysis and oxidation during storage. However, the presence of certain genotypes combining relatively high yield with moderate rancidity suggests that these traits are not strictly antagonistic. For instance, Cluster IV exhibited superior grain yield but comparatively higher rancidity values, whereas Cluster I showed lower rancidity with moderate yield performance. These observations indicate that yield and rancidity traits may be governed by largely independent genetic mechanisms, as also reported in principal component analysis studies where these traits contributed to different components (Rajotia *et al*., 2025).

The relative contribution of traits towards genetic divergence revealed that panicle length (17.97%) contributed the maximum proportion followed by days to flowering (17.06%), plant height (16.78%) and number of productive tillers per plant (16.27%). Grain yield per plant also showed substantial contribution (15.20%) to the total divergence. In contrast, rancidity-related traits such as CAV diff and CPV diff contributed comparatively less. The higher contribution of morphological and yield-related traits towards total divergence suggests their greater role in differentiating the genotypes (Sankar *et al*., 2014; Singh *et al*., 2023). Similar findings have been reported by Shashibhushan *et al*. (2022) and Sankar *et al*. (2014), where morphological traits contributed significantly to genetic divergence. In contrast, the comparatively lower contribution of rancidity-related traits indicates limited variability for these parameters among the genotypes studied. This suggests that selection for reduced rancidity may be comparatively more challenging and requires targeted breeding strategies.

Based on the overall performance for grain yield and rancidity-related parameters, genotypes such as ICMB 92777, HMS 57B, HMS 58B, HMS 64B and ICMB 97111 were identified as promising. These genotypes exhibited relatively higher grain yield along with moderate to lower rancidity indices, making them suitable candidates for improving both productivity and flour shelf life. The results further suggest that although a partial trade-off may exist, simultaneous improvement of yield and storage stability is achievable through appropriate selection strategies.

Conclusion

The present study demonstrated substantial genetic diversity among pearl millet genotypes for agro-morphological, seed vigour and rancidity-related traits. The identification of distinct and genetically divergent clusters provides opportunities for selecting suitable parental combinations in breeding programmes. Genotypes combining higher grain yield with lower rancidity indices were identified as promising candidates for improving both productivity and flour shelf life. These findings highlight the potential of integrating yield and quality traits for developing superior pearl millet varieties with enhanced storage stability.

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