



Research article

Seasonal variation in genetic divergence of single-cut fodder sorghum: A multivariate analysis

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Abstract

Limited knowledge of seasonal genetic divergence in single-cut fodder sorghum (*Sorghum bicolor*), particularly under summer conditions, hinders the identification of stable and superior parental lines. To address this gap, genetic diversity among 55 elite single-cut fodder sorghum genotypes was evaluated at CCS Haryana Agricultural University, Hisar, across the summer and *kharif* seasons of 2024 using 10 morphological, 8 biochemical, and 5 biotic stress-related traits. Multivariate analysis based on Mahalanobis' D² statistics and modified Tocher's clustering revealed substantial genetic variability, grouping genotypes into five clusters during summer and seven during *kharif*. High inter-cluster distances indicated significant genetic divergence, suggesting strong potential for heterotic hybridization. Cluster mean performance and divergence patterns identified key genotypes for targeted breeding, *viz.*, CSV 19 SS for high biomass and micronutrient content (Zn, Fe), SH 2032 and SH 2136 for stable performance across seasons, and CSV 53F, CSV 44F, and HJ 1514 for high yield, better nutritional quality and lower HCN content. Genotype S 490, forming a singleton cluster in the *kharif* season, exhibited resistance against sooty stripe, shoot fly dead hearts and stem borer dead hearts, indicating its potential utility in resilience breeding. Seasonal comparison revealed greater genetic divergence under summer conditions, highlighting its importance for identifying highly discriminating genotypes under stress environments. Overall, the integration of yield, quality, and biotic stress traits across seasons provides a robust framework for selecting diverse and complementary parents. By elucidating genotype divergence across seasons, this study facilitates the selection of superior parental lines to develop climate-resilient, nutrient-rich fodder sorghum for sustainable livestock and bioenergy systems.

Keywords: Biotic stress resistance, Fodder sorghum, Mahalanobis D², Nutritional quality

Introduction

Fodder sorghum (*Sorghum bicolor* (L.) Moench) is an important climate-resilient forage crop valued for its drought tolerance, water-use efficiency, adaptability to marginal environments, and high biomass production under heat and moisture stress (Paterson *et al.*, 2009; Kumari *et al.*, 2024; Seth *et al.*, 2025). In North India, it plays a major role in sustaining livestock-based production systems (Shyamprasad *et al.*, 2021). Breeding efforts in fodder sorghum have focused on improving biomass yield, fodder quality, micronutrient content, and resistance to biotic stresses, leading to the development of several promising breeding lines and cultivars (Kumari *et al.*, 2020; Rajarajan *et al.*, 2021; Dalip *et al.*, 2022). Despite its importance, information on the genetic variability and divergence of single-cut fodder sorghum genotypes under contrasting seasonal environments

remains limited, particularly under summer conditions characterized by high temperature and moisture stress, where adapted varieties are scarce (Behera *et al.*, 2024). Most previous studies have emphasized *kharif*-season evaluation, offering only a partial understanding of genotype performance and diversity across environments (Kumar *et al.*, 2023; Akshitha *et al.*, 2025; Gebre *et al.*, 2025). Multi-season evaluation is therefore essential to identify stable and broadly adapted genotypes while capturing environmentally induced variation in trait expression (Barsalia *et al.*, 2024; Suvadra *et al.*, 2025).

Multivariate approaches such as Mahalanobis' D² statistics provide an effective means to quantify genetic divergence, identify major contributing traits, and classify genotypes into distinct groups for parental selection in breeding programs (Deep *et al.*, 2020; Shafiqurrahman *et al.*, 2024). Accordingly, the present study evaluated 55

elite single-cut fodder sorghum genotypes, including advanced breeding lines and released cultivars, across summer and kharif seasons to assess genetic variability and divergence using multivariate approaches; identify key traits contributing to divergence; and classify genotypes into distinct clusters for selection of diverse parental lines for hybridization.

Materials and Methods

Experimental material and design: The pedigree of the 55-elite single-cut fodder sorghum genotypes is given in Table 1, which were evaluated at CCS Haryana Agricultural University, Hisar, during the summer and

kharif seasons of 2024 (215.52 meters above mean sea level). Hisar is located in a semi-arid sub-tropical zone between 29° and 10° N latitude and 75° and 46° E longitude. The experimental field has deep, alluvial sandy loam soil that is well-drained and capable of retaining a considerable amount of moisture (Kumari *et al.*, 2023).

The study was set up in a randomized block design with three replications, keeping plant-to-plant and row-to-row spacing at 15 and 25 cm, respectively. Each genotype in each block had two rows of two meters in length. Twenty-two traits were recorded on five randomly selected plants per genotype within each block, and the mean across plants and replications was used for subsequent analysis.

Table 1. List of sorghum genotypes included with pedigree.

S. No.	Genotype	Selection from	S. No.	Genotype	Selection from
1	CSV 19 SS	RSSV 2 × SPV 462	29	HJ 1514	SGL 87 × HJ 513-15-10-11-4
2	CSV 21F	GSSV-148 × SR-897	30	HJ 513	S-305 (PJ 7R × SPV 80) × HC 136
3	CSV 30F	NSS 223 × NARI 111	31	HJ 541	SPV 80 × 29/1 (P20-1-1-2)
4	CSV 32F	HC 260 × B 35- 5-3-1-1	32	IS 651	-
5	CSV 35F	PC 5 × IS 7002	33	PC 3	Vidisha 60-1 × Germplasm line IS6953
6	CSV 38F	(CSV 20 × PC 5) × (CSV 20 × PVK 809)- 11-1-1-2-1-1	34	PC 5	CS 3541 × IS 6953
7	CSV 44F	HC 308 × S 437-1-2	35	RSSV 9	RSSV 2 × SPV 462
8	CSV 53F	G 71 × SSG 59-3	36	S 437	S 153 × ROX (P-1-3-7-1-1)
9	G 46	-	37	S 490	S 178 × SPV 394
10	GFS 5	SPV-1087 × GSSV-148	38	S 540	Selection form S 512
11	GJ 39	M49 × M51	39	S 614-13	G 48 × SSG 59 (P-3-4)
12	HBMR 3	PBMR 5 × HJ 541-11-6-7-2	40	SH 1488	SGL 87 × S 241 (P-1)
13	HC 136	IS – 3214 × PJ – 7R	41	SH 1603	SGL 87 × HJ 513 (P-8-9-11-3)
14	HC 260	SPV 103 × PC 9	42	SH 1955	CSV 21F × HJ 541 (P-16-9-8-7)
15	SH 2018	CSV 21F × HJ 541 (P-9)	43	SH 2241	SSG 87 × HJ 541
16	SH 2024	SPV 2185 × HJ 541 (P-3)	44	SH 2243	CSV 21F × IS 2205
17	SH 2032	296A × CSR 30	45	SH 2247	PC 8 × HJ 541
18	SH 2122	CoFS 29 × HJ 541	46	SH 2249	CSV 21F × CoFS 29
19	SH 2136	CSV 21F × HJ 541 (P-12)	47	SH 2251	CSV 21F × CoFS 29
20	SH 2137	CSV 21F × HJ 541 (P-13)	48	SH 2266	HJ 541 × IS 18551
21	SH 2142	HJ 513 × IS 2205 (P-16)	49	SH 2267	HJ 541 × IS 18551
22	SH 2143	IS 23514 × HJ 513 (P-6)	50	SH 2268	HJ 541 × IS 18551
23	SH 2153	SSG 59 × HJ 541 (P-11)	51	SH 2270	PC 8 × CoFS 29
24	SH 2221	CSV 21F × CoFS 29	52	SH 2280	CSV 24SS × IS 18551
25	SH 2231	CSV 24SS × IS 2205	53	SL 45	Chain crossing of Ramkel Sel., RSSV 9 Sel., LC 1, ICSB 94012, LC 2 and SL 44 followed by selection
26	SH 2238	UTMC 539 × HJ 541	54	SPV 2911	PBMR 5 × HJ 541-11-6-7-9
27	SH 2239	SSG 87 × HJ 541			

Parameters analyzed: The traits studied belonged to three major groups: i) morphological and yield traits-Days to 50 per cent flowering (DF; days), plant height (PH; cm), leaves per plant (NL; number), leaf length of the fifth leaf from ground (LL; cm), leaf breadth of the fifth leaf from ground (LB; cm), stem girth (SG; cm), leaf: stem ratio (LSR), green fodder yield (GFY; kg/plot) and dry fodder yield (DFY; kg/plot); ii) quality traits included total soluble solids (TSS; °brix), HCN (µg/g fresh weight), mineral content (Zn, Fe, Cu and Mn; µg/g dm), crude protein (CP; %) and total crude protein yield (CPY; kg/plot). Biotic stress resistance parameters *viz.*, shoot fly (SFDH; %) and stem borer incidence (SBDH; %), grey leaf spot intensity (GLS; %), zonate leaf spot intensity (ZLS; %) and sooty stripe intensity (SS; %). Observations were recorded for pest attack as per Mathur *et al.* (1991) and were classified according to Gomashe *et al.* (2010). Scoring was done using visual standards following Sharma (1978) for leaf spot and sooty stripe in sorghum and further classified based on Marley *et al.* (2001). Additionally, harvesting was done at either 50% flowering or 80 days after sowing, whichever came first; hence, we have only included the data for those genotypes that flowered (at least 50%) on or before 80 days after sowing.

Statistical analysis: To ascertain the genetic divergence among the genotypes, the data were subjected to Mahalanobis D² analysis. As explained by Singh and Chaudhary (1977), D² values were computed for every combination of each genotype. The modified Tocher approach, as implemented by Silva and Dias (2013), was used to arrange the genotypes into various clusters. These analyses were performed using the biotools package (da Silva *et al.*, 2017) in R-4.5.0 software.

Results and Discussion

Analysis of variance: The analysis of variance of 55 elite single-cut fodder sorghum genotypes showed significant genetic variation ($p \leq 0.01$) across morphological, quality, and biotic resistance traits in summer and *kharif*, 2024 (Table 2). To account for correlations between phenotypic traits, we employed Mahalanobis D² statistics (Ujjainkar, 2025). Pairwise genetic distances were estimated for all genotypes and the resulting D² values were subjected to the modified Tocher clustering method, which efficiently grouped genotypes into maximally divergent clusters, *i.e.*, five clusters in summer and seven in *kharif* (Table 3), while maintaining intra-cluster homogeneity as reported by Patil *et al.* (2025), Greeshma *et al.* (2025) and Santhiya *et al.* (2025).

Genetic divergence and cluster analysis: This study confirms the presence of substantial genetic diversity among fodder sorghum genotypes, as reflected in clustering patterns (Table 3) and inter-cluster distances

Table 2. Analysis of variance (ANOVA) showing mean sum square of twenty-two traits studied in summer and *kharif* 2024.

Season	Summer	Kharif
Source of variation	Treatments	Treatments
Degree of freedom	54	54
Days to 50 per cent flowering (DF)	1474.134**	902.278
Plant height (PH)	278.564**	3126.644**
Number of leaves per plant (NL)	12.185**	16.141**
Leaf length (LL)	109.854**	131.754**
Leaf breadth (LB)	0.347**	1.121**
Stem girth (SG)	3.185**	7.531**
Leaf: Stem ratio (LSR)	0.005**	0.006**
Green fodder yield (GFY)	0.116**	0.971**
Dry fodder yield (DFY)	0.008**	0.102**
Total soluble solids (TSS)	1.746**	11.955**
HCN	3398.919**	2916.850**
Crude protein (CP%)	3.195**	3.080**
Total crude protein yield (CPY)	2.077**	4.426**
Copper (Cu)	3.886**	9.030**
Manganese (Mn)	34.069**	43.645**
Iron (Fe)	15217.918**	7083.157**
Zinc (Zn)	116.750**	124.211**
Grey leaf spot intensity (GLS)	27.424**	604.740**
Zonate leaf spot intensity (ZLS)	74.664**	471.644**
Sooty stripe intensity (SS)	10.807**	37.338**
Shoot fly incidence (SFDH)	22.767**	36.306**
Stem borer incidence SBDH)	123.288**	121.214**

** Significant at 1 % level ($p \leq 0.01$); NS Non-Significant

(Fig. 1), representing unique genetic resources that can be utilized to broaden the breeding base and develop superior hybrids, as also reported by Ahalawat *et al.* (2018) and Mulugeta *et al.* (2024). The clustering pattern differed markedly between seasons, with *kharif* 2024 producing a greater number of clusters (seven) than summer 2024 (five), indicating finer phenotypic stratification of genotypes under monsoon conditions. This may be attributed to the more congenial *kharif* environment, characterized by favorable moisture availability, moderate temperatures, and humid conditions, which likely enabled fuller expression of the genotypes' genetic potential across growth, biomass, quality, and micronutrient traits. The humid monsoon environment also favored a greater natural incidence of insects and diseases, thereby increasing variation in biotic stress responses and contributing additional discriminatory traits for clustering. In contrast, the summer season imposed substantial abiotic stress through persistent

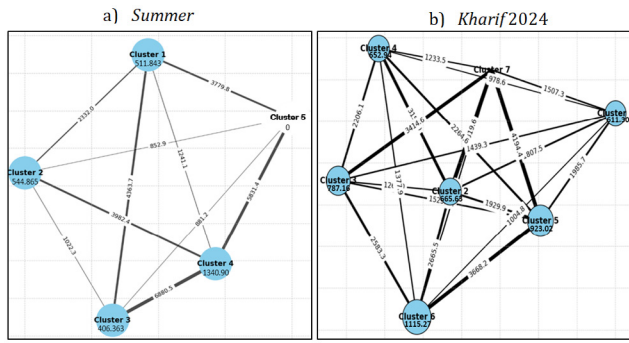


Fig. 1. Cluster diagram of sorghum genotypes across seasons, where node size indicates intra-cluster distance and line thickness represents the distance between groups.

high temperatures and moisture deficit (Fig. 2), which likely constrained the expression of yield and related traits across genotypes. Under such conditions, natural insect and disease pressure was minimal, and phenotypic performance tended to converge toward survival-oriented responses, thereby reducing the number of distinct phenotypic groups and causing clustering to reflect primarily variation in stress adaptation rather than full productive potential. Genotype $\times$ environment (G $\times$ E) interactions and trait plasticity may have further influenced this seasonal clustering pattern (Bahadur *et al.*, 2025; Arunkumar *et al.*, 2014), as certain traits—particularly biotic stress-related traits—exhibited stronger

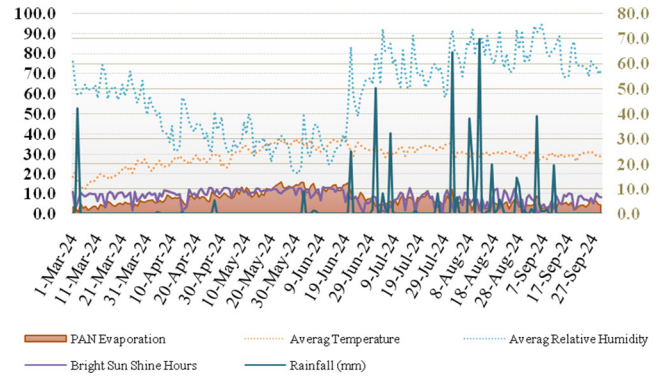


Fig. 2. Dekadal (10-day) trends of key weather parameters recorded during the experimental period. Average temperature (°C) and average relative humidity (%) are on primary Y-axis (dotted lines) whereas, bright sunshine hours (hours), rainfall (mm) and PAN evaporation (mm/day) are on secondary Y-axis (solid lines)

discriminatory power under favorable *kharif* conditions, whereas stress-adaptive traits contributed more prominently under summer stress.

Despite fewer clusters in summer, both intra- and inter-cluster distances (Fig. 1) were consistently greater in summer (intra: 406.36–1340.90; inter: up to 6880.51) than in *kharif* (intra: up to 1115.27; inter: up to 4319.62), indicating enhanced phenotypic divergence among clusters under stress conditions. This suggests that while summer

Table 3. Seasonal variation in cluster membership and genotypic counts in studied population

Cluster No.	Genotypes	No. of Genotypes
Summer 2024		
I	CSV 35F, CSV 38F, CSV 44F, HJ 1514, HJ 513, HJ 541, PC 3, S 437, S 490, S 540, S 614-13, SH 1955, SH 2018, SH 2024, SH 2122, SH 2239, SH 2243, SH 2251, SH 2266, SPV 2911	20
II	G 46, GFS 5, GJ 39, HC 136, HC 260, PC 5, SH 2137, SH 2153, SH 2231, SH 2238, SH 2240, SH 2247, SH 2249, SH 2267, SH 2280	15
III	SH 2143, SH 2270, UTM 1539	3
IV	CSV 21F, CSV 30F, CSV 32F, CSV 53F, HBMR 3, IS 651, RSSV 9, SH 1488, SH 1603, SH 2032, SH 2136, SH 2142, SH 2221, SH 2241, SH 2268, SL 45	16
V	CSV 19 SS	1
Kharif 2024		
I	CSV 19 SS, CSV 21F, CSV 32F, SV 35F, CSV 38F, G 46, GFS 5, GJ 39, IS 651, PC 5, SH 1603, SH 2137, SH 2142, SH 2143, SH 2221, SH 2231, SH 2241, SH 2243, SH 2249, SH 2251, SH 2266, SH 2267, SH 2270, SH 2280, SL 45, SPV 2911, UTM 1539	27
II	CSV 44F, CSV 53F, HC 260, HJ 1514, HJ 513, HJ 541, S 437, SH 1955, SH 2018, SH 2032, SH 2238, SH 2240	12
III	SH 2024, SH 2153, SH 2239, SH 2247, SH 2268	5
IV	CSV 30F, RSSV 9, S 540, SH 2122, SH 2136	5
V	HC 136, S 614-13, SH 1488	3
VI	HBMR 3, PC 3	2
VII	S 490	1

Table 4. Seasonal cluster-wise mean values of agronomic, quality, nutritional, and stress-related traits across forage sorghum genotypes

Traits	Summer 2024					Kharif 2024						
	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII
DFY	0.83	0.88	0.78	0.9	0.93	0.95	1.22	1.05	0.75	0.95	1	0.67
GFY	3.44	3.66	3.25	3.73	3.85	4.2	4.99	4.75	3.64	4.39	4.19	3.16
DF	76.42	74.38	73.50	77.39	-	75.96	74.33	80.00	76.67	80.00	73.00	77.00
PH	178.97	182.25	173.89	183.35	172.11	213.61	270.46	244.87	183.93	247.52	197.28	189.22
NL	10.96	11.47	9.33	12.33	12.45	13.35	16.93	16.07	11	15.15	11.78	11.11
LL	44.07	49.57	40.11	52.49	56.89	66.06	76.06	70.58	58.2	72.41	61.94	62.11
LB	4.91	5.23	5.09	5.22	5.39	6.56	7.52	7.18	6.02	6.93	6.41	6.43
SG	11.13	11.14	11.49	10.66	11.07	16.31	18.7	17.53	15.03	17.25	16.42	15.59
LSR	0.31	0.32	0.31	0.34	0.36	0.29	0.34	0.31	0.25	0.33	0.25	0.22
TSS	4.66	4.33	3.33	4.52	5.07	6.84	6.82	9.88	9.36	11.87	4.51	5.84
HCN	95.88	85.55	94.68	105.49	99.46	81.78	65.63	102.43	98.02	81.7	97.18	134.38
CP%	9.68	7.74	8.06	9.4	7.05	9.13	8.36	7.78	9.64	10.2	8.63	9.84
CPY	0.08	0.07	0.06	0.08	0.07	0.09	0.1	0.08	0.07	0.1	0.09	0.07
Cu	12.23	13.1	12.27	13.85	14.19	15.07	16.76	14.33	14.31	16.19	16.82	12.71
Mn	20.8	22.38	20.86	23.17	22.41	23.6	29.18	22.82	24.54	24.22	26.57	19.39
Fe	148.86	227.88	158.97	227.33	216.32	208.42	252.14	221.78	185.39	232.39	274.21	203.85
Zn	40.3	41.18	42.07	42	43.14	38.41	44.82	43.47	34.63	45.69	32.9	25.83
GLS	12.47	11.6	13.58	12.11	23.46	16.87	12.86	15.06	24.94	16.46	12.96	92.59
ZLS	13.39	13.83	11.52	12.73	35.8	19.25	18.42	15.8	21.73	36.63	16.05	50.62
SS	11.11	11.77	11.11	12.42	11.11	12.94	14.61	11.36	15.31	16.05	12.96	11.11
SFDH	4.93	5.62	4.92	7.51	5.83	13.72	13.31	11.88	12.63	13.14	15.38	11.88
SBDH	18.74	20.59	13.89	20.18	35	28.38	28.54	23.73	26.74	22.06	27	23.13

DFY- Dry fodder yield, GFY- Green fodder yield, DF – Days to 50% flowering, PH- Plant height, NL- No. of leaves per plant, LL- Leaf length, LB- Leaf breadth, SG- Stem girth, LSR- Leaf stem ratio, TSS- Total soluble solids, HCN- Hydrogen cyanide, CP%- Crude protein%, CPY- Total crude protein yield, Cu- Copper content, Mn- Manganese content, Fe- Iron content, Zn- Zinc content, GLS- Grey leaf spot intensity, ZLS- Zonate leaf spot intensity, SS- Sooty stripe intensity, SFDH- Shoot fly incidence, SBDH- Stem borer incidence

stress reduced the number of broad phenotypic groups, it simultaneously amplified differences among the groups that did form, likely through differential expression of adaptive traits such as stress tolerance, biomass partitioning, and nutrient remobilization. Conversely, under the relatively buffered *kharif* environment, although more clusters were formed due to broader trait expression, the magnitude of divergence between those clusters was comparatively lower because genotypes performed under less restrictive and more uniform growing conditions. Nevertheless, the lower divergence observed in *kharif* should not be interpreted as reduced usefulness for evaluation; rather, it reflects greater environmental stability and may support more reliable assessment of yield potential and quality traits, whereas summer may be particularly useful for discriminating

stress-adaptive and highly divergent genotypes. However, confirming these seasonal patterns would require multi-year and multi-environment validation rather than relying on a single seasonal comparison. The observed variation in intra-cluster distances reflects differing levels of genetic heterogeneity within clusters, arising from the grouping of genotypes that may share overall similarity yet differ in specific trait combinations. Inter- and intra-cluster distances, when interpreted alongside cluster mean performance, therefore provide a robust framework for identifying genetically divergent and agronomically superior parental combinations for sorghum improvement. For instance, Cluster IV in summer, which exhibited the highest intra-cluster distance (1340.90), also recorded superior mean performance for DFY, GFY, PH, CPY and micronutrient

Table 5. Classification of sorghum genotypes based on cluster consistency and adaptive stability across summer and *kharif* 2024.

Genotype	Cluster membership		Remarks
	Summer	Kharif	
CSV 35F, CSV 38F, SH 2243, SH 2251, SH 2266, SPV 2911	1	1	They are homeostatic and reliable regardless of environmental shifts hence may be considered as core breeding material for stable fodder production.
HC 260, SH 2238, SH 2240	2	2	
CSV 30F, RSSV 9, SH 2136	4	4	
CSV 44F, HJ 1514, HJ 513, HJ 541, S 437, SH 1955, SH 2018	1	2	These genotypes, though don't fall apart under changing environment, but shift their resource allocation to match the season. These genotypes show balanced adaptation.
SH 2024, SH 2239	1	3	
S 540, SH 2122	1	4	
G 46, GFS 5, GJ 39, PC 5, SH 2137, SH 2231, SH 2249, SH 2267, SH 2280	2	1	
SH 2153, SH 2247	2	3	
SH 2143, SH 2270, UTM 1539	3	1	
CSV 21F, CSV 32F, IS 651, SH 1603, SH 2142, SH 2221, SH 2241, SL 45	4	1	
CSV 53F, SH 2032	4	2	
CSV 19 SS, HBMR 3, HC 136, PC 3, S 490, S 614-13, SH 1488, SH 2268	No common cluster across seasons		

(Cu, Mn, Fe and Zn) content (Table 4), indicating that the cluster contains both high- and moderately-performing genotypes with diverse trait architectures, thereby inflating within-cluster distance. In contrast, clusters with lower intra-cluster values, such as Cluster III, are comparatively more homogeneous, suggesting tighter grouping of genotypes with similar phenotypic profiles. The maximum inter-cluster distance during summer between Clusters III and IV (6880.51) indicates substantial divergence driven by contrasting multivariate trait profiles—Cluster IV being characterized by high biomass yield and micronutrient content, whereas Cluster III comprises genotypes with superior biotic resistance traits. Such divergence typically arises when clusters differ simultaneously across several correlated structural, physiological, and quality-related attributes (e.g., yield vs. quality trade-offs, structural vs. physiological attributes), resulting in large Mahalanobis distances. Because Cluster IV combines superior mean performance with internal heterogeneity, while Cluster III is relatively uniform and contrasting, crosses between these clusters offer strong potential for exploiting heterosis and generating transgressive segregants.

A similar interpretation applies in *kharif*, where Cluster VI showed the highest intra-cluster distance (1115.27), indicating substantial heterogeneity, but Cluster II emerged as the most promising based on cluster mean performance (Table 4), exhibiting superior yield, morphological traits and micronutrient concentrations

together with lower HCN content. This distinction emphasizes that high intra-cluster distance reflects heterogeneity rather than agronomic superiority *per se*. The maximum inter-cluster distance in *kharif* occurred between Clusters II and VII (4319.62); however, Cluster VII comprised a single genotype (S 490), and despite its genetic distinctness, its undesirable trait profile—including high HCN, poor yield and quality traits—limits its immediate value as a direct parent for general fodder improvement. Accordingly, such highly distinct singleton genotypes should be considered cautiously and utilized primarily for targeted introgression of specific beneficial traits such as resistance to sooty stripe and shoot fly dead hearts, *i.e.*, traits that are validated, rather than broadly recommended for hybridization solely on the basis of genetic distance.

Seasonal shifts in cluster membership and the emergence of singleton clusters further underscore substantial G×E interaction within the studied germplasm. The formation of unique singleton clusters in specific seasons suggests that certain genotypes possess highly distinct functional responses to seasonal environmental conditions. For example, CSV 19 SS— the only sweet sorghum genotype included in the study—formed a singleton cluster during summer (Cluster V), likely due to its unique sweet sorghum genetic architecture, which prioritizes sugar accumulation and leaf density, thereby associated with elevated TSS, high leaf:stem ratio, and enhanced micronutrient (Cu, Zn) accumulation *albeit* at the cost

of increased susceptibility to biotic stressors like stem borer and zonate leaf spot (Table 4), distinguishing it clearly from conventional forage sorghum entries. Furthermore, CSV 19 SS is not specifically adapted to the test location, which may also contribute to its unique performance and isolation within the cluster analysis. Conversely, the singleton clustering of S 490 during *kharif* reflects pronounced phenotypic distinctness under monsoon conditions due to its relative lack of adaptive plasticity compared to the rest of the population, and its agronomic limitations necessitate cautious breeding use. Additionally, genotypes maintaining consistent cluster membership across seasons (Table 5) likely possess greater phenotypic stability and environmental buffering capacity, making them attractive candidates for breeding superior genotypes with improved yield, quality and tolerance to biotic stress. Conversely, genotypes exhibiting major seasonal shifts in cluster assignment or no common cluster membership across seasons (e.g., HBMR 3, HC 136, PC 3, S 614-13) demonstrate high phenotypic plasticity and may serve as valuable donors of adaptive alleles for season-specific or stress-targeted breeding. Notably, despite its seasonal shift in cluster placement, HC 136 has been widely recognized as a stable and broadly adapted genotype since its release in 1986, consistently performing well across locations in *kharif* for biomass and forage quality. This suggests that its cluster reassignment may reflect dynamic trait expression under varying environments rather than instability *per se*, highlighting its value as a donor for wide adaptability and robust performance in breeding programs. Similar patterns have also been reported in multi-environment sorghum studies, where certain genotypes exhibit high stability across environments while others shift cluster associations depending on seasonal conditions, reflecting differential adaptability (Demelash, 2024; Birhanu *et al.*, 2024; Kumari *et al.*, 2025).

Overall, integrating divergence metrics with cluster mean performance enables a more refined and biologically meaningful parental selection strategy by balancing genetic distance, trait complementarity, and environmental stability. Based on the present results, Clusters III and IV in summer offer the greatest potential for generating heterotic and transgressive segregants for biomass and nutritional improvement. For biomass enhancement specifically, Cluster IV (summer) and Cluster II (*kharif*; e.g., CSV 53F, HJ 1514, SH 2032) represent key donor pools due to their superior agronomic performance, whereas clusters such as V (summer; CSV 19 SS) and III provide useful variation for quality-related improvement, including micronutrient enrichment. However, caution is warranted when interpreting distances involving clusters with very few genotypes, particularly singleton clusters, as their breeding utility should be judged by trait-specific merit rather than

divergence alone. Collectively, these findings suggest that *kharif* and summer should be viewed as complementary evaluation environments: *kharif* for stable assessment of yield, quality, and biotic stress traits under favorable conditions, and summer for identifying stress-resilient, highly divergent parental lines. Nevertheless, these seasonal patterns should be validated across multiple years and environments before establishing definitive season-specific breeding strategies.

Conclusion

This study found substantial genetic variability among elite single-cut fodder sorghum genotypes and elucidated its structure across contrasting summer and *kharif* environments. The evaluation also facilitated the delineation of superior and complementary genotypes for targeted improvement. Genotypes such as SH 2032, SH 2136, CSV 53F, and CSV 44F demonstrated consistent performance across seasons, indicating their suitability as stable parental lines. In contrast, genotypes like CSV 19 SS and S 490, characterized by unique trait profiles and cluster positions, represent valuable sources of specific attributes, including high biomass, micronutrient enrichment, and stress-associated traits. Notably, the summer-season evaluation provided critical insights into genotype performance under stress-prone conditions, addressing the limited availability of adapted varieties and trait information for this season. By integrating morphological, biochemical, and biotic stress resistance traits across environments, this study offers a comprehensive understanding of genotype performance and adaptability. The findings support the strategic selection of diverse and complementary parents (CSV 53F, HJ 1514, SH 2032, CSV19SS and S 490) for developing season-adapted, climate-resilient fodder sorghum varieties and hybrids.

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