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## GENETIC DIVERSITY FOR FALL ARMYWORM (*SPODOPTERA FRUGIPERDA*) RESISTANCE AND PRODUCTIVITY TRAITS IN SORGHUM (*SORGHUM BICOLOR* L. MOENCH) GERMPLASM

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### ABSTRACT

An investigation was conducted to dissect the genetic divergence in a panel of 278 sorghum genotypes, including minicore accessions and improved lines, under natural fall armyworm (FAW) infestation. Application of Mahalanobis D<sup>2</sup> statistics and Tocher's clustering grouped the genotypes into 15 clusters, with Cluster I as the largest (147 genotypes) and Clusters XII, XIII, XIV, and XV representing solitary accessions. Maximum intra-cluster diversity was observed in Cluster XI (34.68), while the most pronounced inter-cluster divergence was between Cluster XII and Cluster V (422.68), revealing considerable genetic differences among clusters and underscoring the potential for hybridization between distant clusters to exploit maximum variability. Principal component analysis identified three major components contributing 61.3% of the total variance. Trait-specific cluster means highlighted distinct genetic resources: Cluster XI harboured the promising FAW-resistant and tallest plants, Cluster VI excelled in grain yield, Cluster XII showed superior seedling vigour, Cluster IX demonstrated enhanced glossiness, and Cluster III represented genotypes with the lowest leaf sheath pigmentation. Analysis of divergence showed that plant height contributed 41.2% to total genetic divergence, followed by leaf sheath pigmentation (34.2%) and grain yield (17.3%), with these three traits together accounting for over 90% of diversity among the genotypes. These findings provide a robust framework for breeding programs, advocating hybridization between genetically diverse clusters, especially those with outstanding performance for key resistance and agronomic traits, to develop high-yielding, FAW-resilient sorghum cultivars for sustainable agriculture in pest-prone environments.

**Keywords :** Sorghum, Fall armyworm, Genetic divergence, Cluster, Principal component.

### Introduction

Sorghum (*Sorghum bicolor* (L.) Moench), a globally important cereal crop, is valued for its diverse applications as food, feed, fodder, and fuel. Its resilience to drought, heat, and low-input conditions makes it particularly suited for cultivation in climate-stressed, water-scarce regions, directly contributing to food and nutritional security (Hossain *et al.*, 2022). Despite its importance, sorghum productivity is

increasingly constrained by insect pests, which cause economic losses exceeding US\$1 billion annually in the semi-arid tropics (Arora *et al.*, 2025; Onkarappa *et al.*, 2024). Among these, the fall armyworm (FAW, *Spodoptera frugiperda* J.E. Smith) has emerged as a highly invasive pest, capable of inflicting yield losses of up to 80% in maize, sorghum, and millets, with estimated economic damage of \$13 billion annually in sub-Saharan Africa (Overton *et al.*, 2021).

Host plant resistance (HPR) offers one of the most sustainable and farmer-friendly strategies for FAW management (Deshmukh *et al.*, 2021; Prasanna *et al.*, 2018). However, FAW resistance in sorghum remains poorly studied compared to maize. Since resistance is quantitative and polygenic in nature, the presence of adequate genetic variability in germplasm collections is critical for identifying durable sources of resistance. The sorghum minicore collection (242 accessions) developed at ICRISAT (Upadhyaya & Ortiz, 2001), along with elite breeding lines from the All India Coordinated Research Project (AICRP) on Sorghum, represents a valuable platform for resistance screening and diversity assessment.

Knowledge of genetic variability, heritability, and genetic divergence is essential for effective utilization of germplasm in breeding programs (Johnson *et al.*, 1955). Statistical approaches such as Mahalanobis D<sup>2</sup> analysis and clustering methods enable the quantification of genetic diversity, the classification of genotypes into distinct groups, and the identification of diverse parents for use in hybridization programs. Despite the growing threat of FAW, systematic studies combining genetic variability and clustering analyses in sorghum under natural infestation conditions are limited.

Therefore, the present investigation was undertaken to assess genetic variability, heritability, and genetic divergence among diverse sorghum genotypes evaluated under natural FAW infestation, with the objective of identifying promising and diverse genotypes for their potential use in resistance breeding.

## Materials and Methods

The present investigation was carried out during the post-rainy season of 2023 under natural FAW infestation at the Main Agricultural Research Station (MARS), University of Agricultural Sciences (UAS), Dharwad, Karnataka, India (15°29'N, 74°59'E, 689 m amsl). The experimental material comprised a wide range of sorghum genotypes, specifically 242 minicore accessions from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), 34 elite improved lines curated by the All India Coordinated Research Project (AICRP) on Sorghum, Dharwad, and two common standard checks (resistant: IS 18551; susceptible: Swarna). The trial was laid out in a randomized complete block design with two replications. Each genotype was planted in a single row of 4 m length with a spacing of 75 cm between rows and 15 cm between plants. Thinning was carried out 10 days after emergence (DAE) to establish a final stand of 25–26 plants per plot. To encourage uniform natural

infestation of FAW, a border row of maize was planted around the experimental plots.

Data were recorded from five randomly selected plants for six quantitative traits namely foliar damage score, leaf glossiness, seedling vigour, leaf sheath pigmentation, plant height (cm), and grain yield per plant (g). Foliar damage (FDS) was scored visually at 40 DAE on a 0–9 scale (0 = no visible damage, 9 = complete defoliation) as per (Davis *et al.*, 1992; Wiseman *et al.*, 1966). Leaf glossiness (GLS) was assessed visually at 10 DAE on a 1–3 scale (1 = highly glossy, 3 = non-glossy), and seedling vigour (VGR) was scored on a similar 1–3 scale (1 = highly vigorous, 3 = poor) following (Sharma & Nwanze, 1997). Leaf sheath pigmentation (LSP) was rated at 5–7 DAE on a 1–5 scale (1 = dark pink pigment, 5 = green pigment) as per (Dhillon *et al.*, 2005). Plant height (PH) was measured from the soil surface to the panicle tip at maturity, and grain yield per plant (GY) was recorded from five randomly selected panicles harvested from each plot.

All data analysis was carried out using the 'metan' R package (Olivoto & Lúcio, 2020) in R software. Mahalanobis' D<sup>2</sup> statistics (Mahalanobis, 1936) was employed to quantify the degree of genetic divergence and Tocher's method was used for clustering the genotypes into distinct groups as described by (Rao, 1952). To visualize patterns of genetic divergence, a heatmap of pairwise Mahalanobis D<sup>2</sup> distances was generated, where higher values indicate greater divergence among genotypes. Additionally, hierarchical clustering using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) (Sneath and Sokal, 1973) based on Mahalanobis D<sup>2</sup> distances was performed.

## Results and Discussion

### Basic Statistics

Analysis of variance revealed highly significant genotypic variation ( $p < 0.01$ ) (Table 1) for most traits, indicating the presence of distinct genetic control patterns across them. LSP, PH, and GY exhibited high genotypic variances and broad-sense heritability ( $h^2 = 0.88, 0.90, \text{ and } 0.77$ , respectively). The high genetic advance as a percentage of mean (GA%) for PH (72.39%) and GY (20.99%) further suggests the predominance of additive gene action, implying that direct selection would be effective for these traits (Faysal *et al.*, 2022).

In contrast, VGR, GLS, and FDS showed relatively low heritability ( $h^2 = 0.15\text{--}0.31$ ) and moderate GA, indicating greater environmental influence and non-additive gene effects. Therefore,

improvement of these traits would benefit more from integrated breeding approaches (Yadav *et al.*, 2021).

Principal component analysis based on six traits revealed three major components explaining 61.3% of the total variance, with PC1 (22.8%) and PC2 (20.7%) capturing most of the phenotypic differentiation among genotypes. These components indicate that a few key traits contribute considerably to genetic variability and can guide selection for diversity and trait improvement (Greenacre *et al.*, 2022).

### Cluster Analysis by Mahalanobis Distance

#### Clustering Pattern

Mahalanobis  $D^2$  statistics were used to assess genetic divergence among 278 sorghum genotypes, and clustering was performed using Tocher's method (Rao, 1952). The genotypes were grouped into 15 distinct clusters, revealing considerable variability across traits (Figure 1). Boxplots clearly show wide inter-cluster variation, indicating strong genetic diversity among genotypes. Cluster I was the largest, comprising 147 genotypes, followed by Cluster III (39) and Cluster II (32). Clusters IV–VII contained 7–17 genotypes, while Clusters VIII–XI included 2–5 genotypes each. The remaining Clusters XII–XV were mono-genotypic/solitary, representing highly divergent lines.

The coexistence of large and small clusters reflects varying degrees of genetic relatedness among genotypes. Larger clusters denote genetically similar lines, whereas mono-genotypic clusters represent unique genotypes possessing distinct trait combinations or adaptive advantages. The absence of a clear association between cluster composition and geographical origin suggests that genetic divergence is governed more by selection and adaptation than by geographic distribution (Tiwari *et al.*, 2022).

From a breeding perspective, crossing genotypes from distantly related clusters (Cluster I  $\times$  Cluster XII or Cluster IV  $\times$  Cluster XIII) is expected to generate maximum heterosis and broader genetic variability, thereby enhancing the chances of identifying superior segregates for FAW resistance and yield improvement (Parameshwarappa *et al.*, 2011). The Mahalanobis  $D^2$  heatmap (Figure 2) further highlighted a wide genetic divergence, with clearly distinguishable groups of genotypes separated by higher pairwise  $D^2$  distances (yellow–orange zones). The clustering pattern corresponds well with the Tocher grouping, supporting the presence of considerable genetic variability among the diverse germplasm lines evaluated for FAW resistance and productivity traits. To visualize the genetic divergence pattern, hierarchical cluster analysis (Unweighted Pair Group Method with Arithmetic

Mean) based on Mahalanobis  $D^2$  statistics was performed (Figure 3). The dendrogram revealed clear separation among genotype groups, which broadly corresponded with the clusters obtained through Tocher's method.

#### Intra and Inter-Cluster Distance

The intra and inter-cluster  $D^2$  values among the fifteen clusters are presented in Table 2. Considerable variation was observed in the magnitude of genetic divergence, confirming wide genetic diversity among the genotypes.

The maximum intra-cluster distance was observed for Cluster XI (34.68), followed by Cluster III (27.60) and Cluster VIII (26.65), indicating greater heterogeneity among the genotypes within these clusters. In contrast, the least intra cluster distance (0.00) was recorded for Clusters XII, XIII, XIV, and XV, reflecting a high degree of genetic homogeneity, likely because they contained only one genotype each. Clusters with low intra-cluster distances (II, V, VI, VII, X) suggest that their genotypes are genetically similar, showing a narrow range of variability. Variation in intra-cluster distance values arises from differences in the extent of genetic relatedness and heterogeneity within clusters. Larger intra-cluster distances indicate that genetically diverse genotypes are grouped together, while smaller or zero distances suggest uniformity or mono-genotypic clusters.

A wide range of inter-cluster distances was also observed, highlighting genetic distinctness among genotypes. The maximum inter cluster distance occurred between Cluster XII and Cluster V (422.68), followed by Cluster XII and Cluster IV (369.70), Cluster XIV and Cluster X (363.24), and Cluster XIII and Cluster IV (300.33). These large distances indicate high genetic divergence, suggesting that crosses among these clusters would likely yield superior recombinants and broader genetic variability (Bekis *et al.*, 2021).

Conversely, the lowest inter-cluster distance was observed between Cluster VII and Cluster XIV (64.75) and between Cluster I and Cluster II (66.89), indicating close genetic relationships and limited divergence among these clusters. Crosses among such closely related genotypes may not result in better heterosis or wider variability in progeny (Parameshwarappa *et al.*, 2011).

In this study, the inter-cluster distances were found to be greater than the intra-cluster distances, indicating significant genetic diversity among the mini core accessions. This suggests a higher potential for obtaining rare, superior segregants through crosses

between genetically distant accessions. These findings are consistent with observations reported by previous studies (Gebre *et al.*, 2025; Karadi & Kajjidoni, 2019; Prasad & Biradar, 2018; Santhiya *et al.*, 2025; Verma *et al.*, n.d.; Vijaylaxmi *et al.*, 2019).

### Cluster Means across Traits

The cluster mean values showed wide variation across all traits (Table 3). Cluster III recorded the lowest mean for LSP which has been associated with tolerant or moderately resistant lines. Cluster XII exhibited the lowest mean for VGR, reflecting superior early growth. Cluster IX showed the lowest mean for GLS, indicating enhanced leaf surface reflectance and potential stress tolerance. Cluster XI had the lowest mean for FDS, signifying better resistance to fall armyworm.

For PH, Cluster XI recorded the highest mean, denoting tall and vigorous genotypes suitable for fodder or biomass use. GY was highest in Cluster VI, followed by Clusters II and V, highlighting groups with superior productivity potential. Hence, hybridization among genetically diverse clusters excelling in different traits would help obtain transgressive segregates combining desirable attributes for sorghum improvement.

### Contribution of Traits to Divergence

The contribution of individual traits to total genetic divergence (Figure 4) revealed that PH was the

most influential, contributing 41.2% of total divergence, followed by LSP (34.2%) and GY (17.3%). These three traits together accounted for over 90% of the total variation, highlighting their importance in differentiating genotypes for growth and yield potential.

In contrast, GLS, VGR, and FDS contributed minor but notable proportions (2.9%, 2.3%, and 2.2%, respectively), suggesting their secondary yet relevant roles in shaping the overall diversity structure.

### Conclusion

This comprehensive multivariate assessment of sorghum germplasm under natural FAW infestation revealed significant genetic variability and distinct divergent clusters. Plant height, leaf sheath pigmentation, and grain yield accounted for the majority of genetic divergence, while mono-genotypic clusters identified highly unique and potentially valuable lines. The substantial inter-cluster genetic distances observed suggest that hybridization between genetically distant groups can maximize recombination and unlock superior segregates for FAW resistance and yield enhancement. The study provides a framework for sorghum breeding, guiding the targeted use of genetically diverse parents to achieve resistance breeding goals in the context of global pest pressures and climate resilience.

**Table 1:** Analysis of variance (ANOVA), variance components, broad-sense heritability ( $h^2$ ), and genetic advance (GA) for six traits evaluated under field conditions in sorghum minicore accessions.

Trait	Replication F	Genotype F	Env. Var ( $\sigma_e^2$ )	Gen. Var ( $\sigma_g^2$ )	Phen. Var ( $\sigma^2$ )	$h^2$ (broad)	GA (%)
LSP	14.50***	16.28***	0.21	1.63	1.85	0.88	2.48
VGR	81.69***	1.36**	0.43	0.07	0.51	0.15	0.22
GLS	42.98***	1.88***	0.22	0.09	0.32	0.30	0.36
FDS	35.39***	1.30*	0.74	0.11	0.86	0.13	0.25
PH	182.28***	19.17***	150.92	1,370.95	1,521.88	0.90	72.39
GY	1.79n.s.	7.55***	41.39	135.60	177.00	0.76	20.99

(\*\*\*p < 0.001, \*\*p < 0.01, p < 0.05, n.s. – non-significant)

Leaf sheath pigmentation (LSP), seedling vigour (VGR), glossiness (GLS), foliar damage score (FDS), plant height (PH), and grain yield (GY)

**Table 2:** Average intra (Bolded Diagonal) and inter-cluster (off Diagonal) distances in sorghum diverse germplasm

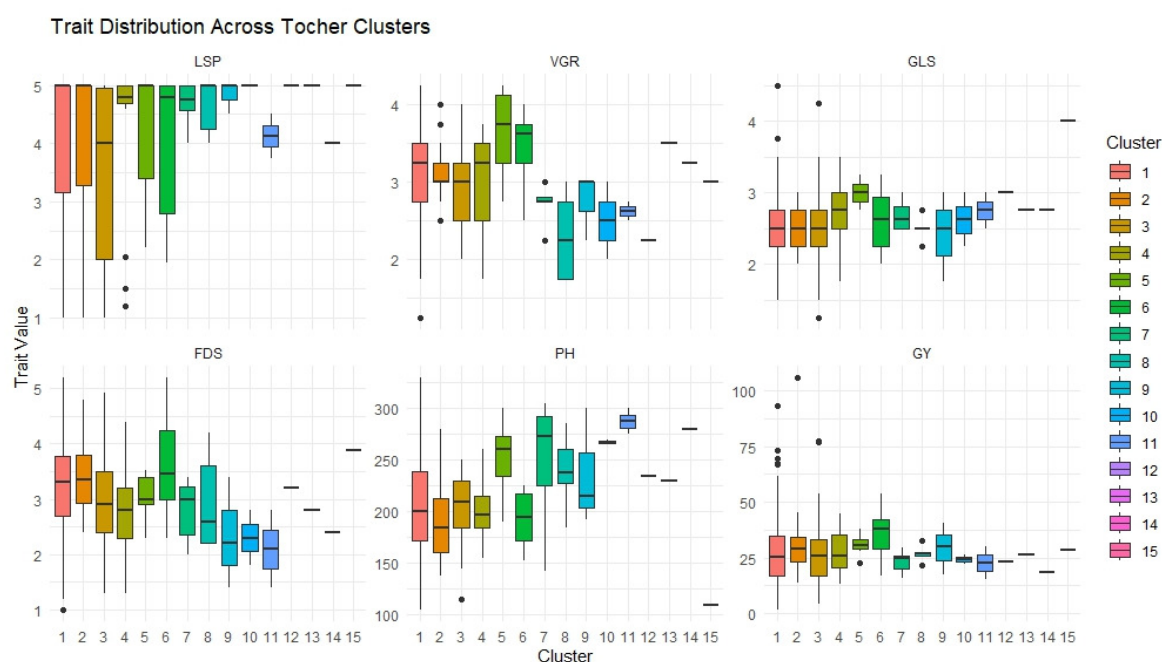
Cluster	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
I	<b>23.01</b>	66.89	83.74	61.28	160.02	94.76	81.19	62.18	40.37	108.25	132.51	205.07	200.59	193.57	120.59
II		<b>19.09</b>	148.48	184.88	301.44	72.21	74.75	153.31	59.24	138.10	157.12	103.60	173.65	229.02	197.10
III			<b>27.60</b>	78.39	58.47	66.98	141.20	82.13	79.51	38.90	59.07	247.20	194.82	260.44	99.76
IV				<b>25.14</b>	82.54	168.41	171.75	60.36	90.01	123.75	161.25	369.70	300.33	263.18	128.86
V					<b>18.99</b>	172.39	250.02	105.74	177.51	96.53	111.02	422.68	300.76	334.90	122.74
VI						<b>21.51</b>	91.74	130.32	78.17	56.76	49.79	107.55	121.40	237.11	128.83
VII							<b>20.17</b>	78.06	123.12	185.26	111.20	68.13	55.45	64.75	67.59
VIII								<b>26.65</b>	116.54	149.84	102.09	224.60	144.56	111.36	38.60

IX									22.56	69.56	146.33	222.58	246.40	284.61	183.40
X									17.14	84.68	260.38	249.94	363.24	177.70	
XI										34.68	149.80	100.42	204.30	73.05	
XII										0.00	47.19	147.80	175.87		
XIII											0.00	59.67	65.46		
XIV												0.00	59.22		
XV													0.00		

**Table 3:** Cluster means for six characters in sorghum diverse germplasm

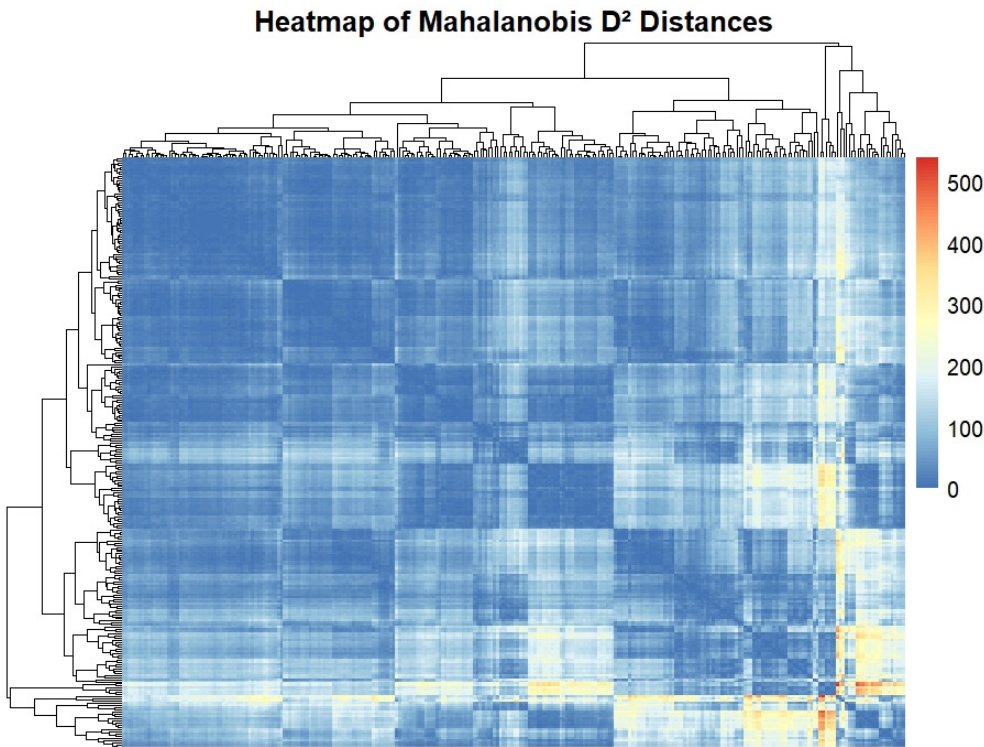
Cluster	LSP	VGR	GLS	FDS	PH (cm)	GY (g)
I	4.05	3.13	2.58	3.21	204.0	28.0
II	4.04	3.09	2.54	3.36	190.0	31.2
III	3.54	2.88	2.49	2.93	205.0	28.3
IV	4.29	3.01	2.69	2.81	200.0	28.3
V	4.14	3.64	3.00	3.06	252.0	30.9
VI	3.98	3.48	2.60	3.61	192.0	35.8
VII	4.70	2.75	2.69	2.81	253.0	23.4
VIII	4.65	2.30	2.50	2.96	239.0	26.9
IX	4.83	2.75	2.42	2.33	236.0	29.4
X	5.00	2.50	2.62	2.30	268.0	24.2
XI	4.12	2.62	2.75	2.10	288.0	22.6
XII	5.00	2.25	3.00	3.20	235.0	23.0
XIII	5.00	3.50	2.75	2.80	230.0	26.6
XIV	4.00	3.25	2.75	2.40	280.0	18.7
XV	5.00	3.00	4.00	3.88	110.0	28.4

Leaf sheath pigmentation (LSP), seedling vigour (VGR), glossiness (GLS), foliar damage score (FDS), plant height (PH), and grain yield (GY)

**Fig. 1 :** Trait distribution across Tocher clusters for six traits in sorghum minicore accessions.

Boxplots depict the variation in leaf sheath pigmentation (LSP), seedling vigour (VGR), glossiness (GLS), foliar damage score (FDS), plant height (PH), and grain yield (GY) among 15 clusters derived from

Mahalanobis  $D^2$  analysis. Each box represents the interquartile range within clusters, showing distinct trait patterns across genotypic groups.

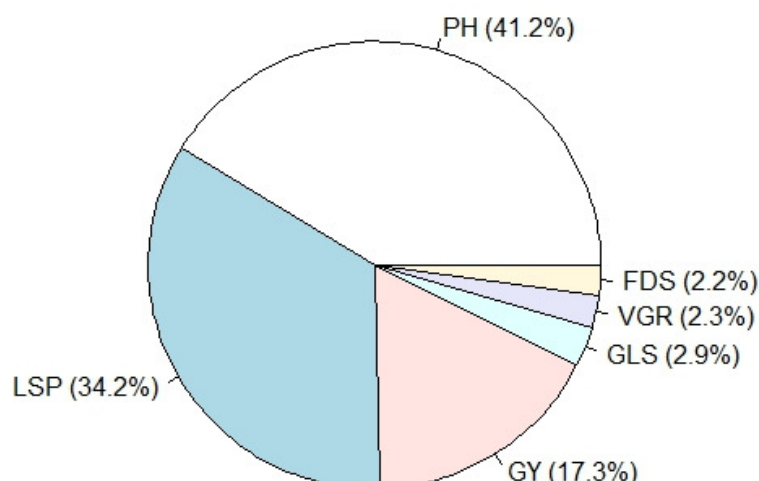


**Fig. 2:** Heatmap of Mahalanobis D<sup>2</sup> distances depicting genetic divergence among sorghum genotypes.

The color gradient represents pairwise D<sup>2</sup> values, where lighter shades (yellow–orange) indicate greater genetic distances, and darker shades (blue) indicate higher similarity among genotypes.



**Fig. 3 :** Hierarchical cluster dendrogram of sorghum genotypes based on Mahalanobis D<sup>2</sup> statistics using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA).



**Fig. 4:** Relative contribution (%) of traits to total genetic divergence among sorghum genotypes based on Mahalanobis  $D^2$  statistics.

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