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## D<sup>2</sup> analysis and principal component analysis to assess genetic divergence in *Sorghum bicolor* (L.) moench for yield and its related attributes

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### Abstract

The present study was carried out during *kharif* 2022-23 at Crop Research Centre-1, Department of Genetics and Plant Breeding, ITM University, Gwalior (M.P.) India. The study was carried out in randomized block design in three replications to evaluate the genetic divergence in 30 sorghum genotypes for 14 characters to determine genetic diversity using D<sup>2</sup> analysis and principal component analysis, D<sup>2</sup> values ranged between D<sup>2</sup> = 80.0061 and D<sup>2</sup> = 3471544.8770 showing high genetic divergence between the genotypes, on the basis of the D<sup>2</sup> values 30 genotypes were grouped into 7 Clusters, Cluster-I with highest 14 genotypes and Cluster-VII with least 1 genotype, inter-Cluster values ranged between D<sup>2</sup> = 3171872.80 to D<sup>2</sup> = 80396.26, while the intra-Cluster values ranged between D<sup>2</sup> = 27422.98 to D<sup>2</sup> = 2032.887 genetic divergence was recorded maximum for transpiration rate (99.1%) on the other hand principal component analysis showed Eigen values > 1 for first four principal components which altogether contributed to 77% of the total genetic divergence.

**Keywords:** Sorghum, D<sup>2</sup> analysis, principal component analysis, transpiration rate

### Introduction

Sorghum [*Sorghum bicolor* (L.) Moench, 2n = 2x = 20] is one of the important staple crops, in African countries which provides food security to areas where water for agriculture is a deficit commodity. For such areas importance of sorghum can be established by the fact that extensive work is being perused in the forms of breeding programs designed for sorghum in such agroclimatic zones, in the present times when 42% of India's land area is under drought crops like sorghum are nature's gift to mankind, which can be further enhanced by breeding. In present India ranks sixth for sorghum production in world with a total production of 4423 thousand tons on 4584 thousand ha of area with a productivity of 1.0 tons/ha (Anonymous, 2022-2023), the ability of sorghum to tolerate drought comes from how its morphology behave when exposed to stress, in addition to it sorghum is also a multipurpose crop as it provide fodder for dairy industry and sweet sorghum varieties are potential substitute for sugar and biofuel production, also Sorghum is good substitute to fulfil the requirement of healthy staple food and fodder in dairy industry, sorghum is important crop in various aspects and should be worked on, for which we have to exploit the variability present for the crop, in the present experiment research scholar has focused on the yield and its related attributes for assessing the genetic divergence using D<sup>2</sup> statistics as for any crop improvement program the knowledge of genetic diversity is a prerequisite the crossing between diverse parent gives heterotic hybrids with good scale of variability which can be evaluated for selection and improvement of various characters as Diversity in germplasm is important for any breeding program, since it directly affects the potential for genetic gain through selection (Kotal *et al.*, 2010) [1].

The principal component analysis was also conducted to differentiate the genotypes on the basis of similarities found among them for various characters as it gives us a clear view of the reason behind the grouping of various genotypes in same group. The high level of genetic diversity and characterization of accessions integrated into world collections is essential in order to classify, manage exotic germplasm, collect and ultimately utilize the different genetic improvement of the crop (Karadi and Kajjiodoni, 2019) [2].

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Objective of the above study is to assess the genetic diversity in sorghum to create ample number of variable sources to exploit the variability in sorghum for development of useful sorghum varieties which can be cultivated commercially in the drought prone areas with significant yields.

### Materials and Methods

The present experiment was conducted in *kharif* season 2022-23 at Crop Research Centre, ITM University, Gwalior (M.P.) India which falls under the Gird agroclimatic region of Madhya Pradesh, with 800-1000 mm of annual rainfall and red, light shallow soil, the experimental site is located at 26° 08' 22.6" N latitude and 78° 11' 42.9" E longitude at a height of 211.5m above sea level. It has a semi humid and subtropical climate. Sowing was done by dribbling method at a spacing of 45cm X 15cm in an area of 300 m<sup>2</sup>, the study was conducted in randomized block design with three replications all the characters were recorded for five plants in each replication and their average was worked out Under the study 30 sorghum genotypes were studied for characters namely Days to 50% flowering, Days to maturity, Leaf area (cm<sup>2</sup>), Plant height at maturity (cm), Stomatal count/microscopic field, Transpiration rate upper surface ( $\mu\text{cm}^{-1}\text{s}^{-1}$ ), Leaf temperature (°C), Number of leaves/plant, Flag leaf length (cm), Length of panicle at maturity (cm), Test weight (g), Green fodder yield/plant (g), Dry fodder yield/plant (g) and Grain yield/plant (g) and subjected to the statistical analysis, the significance of difference between sorghum genotypes was based on wilk's ( $\Lambda$ ) criterion (Wilk, 1932) [3] following which Cluster distances were estimated and genotypes were grouped into population constellations using Tocher's method as described by Rao (1952) [4]. All the statistical analysis was estimated using free source software R Studio, version 4.2.2 package of analysis, for principal component analysis package named 'PRCOMP' was used, all the visualizations were also created using the same software.

### Results and Discussion

Analysis of variance and wilk's lambda showed the genotypes being highly significant following which D<sup>2</sup> statistics analysis by Mahalanobis and Tocher's method was conducted on the 30 sorghum genotypes which grouped all the genotypes into 7 Clusters as mentioned below in Table-1, Cluster-I was grouped with maximum 14 members while the Cluster-VII consisted of only one genotype and Cluster-III with four members followed by which Cluster-II, Cluster-IV and Cluster-VI consisted of three members each and Cluster-V with two members. The groups with a smaller number of members are less diverse which can be because of the same ancestry from which they have been evolved. As summarized in Table-2 Intra Cluster distances for these seven Clusters ranged between 2032.887 to 27422.98 for Cluster II and Cluster IV respectively and Cluster III with D<sup>2</sup> value of 22327.78 was second highest followed by Cluster VI (D<sup>2</sup> = 19041.46), Cluster I (D<sup>2</sup> = 10901.71) and Cluster V (D<sup>2</sup> = 5545.897) intra Cluster distance for Cluster VII was zero because Cluster VII consisted of only one member while the Inter Cluster distances were highest between Cluster I and Cluster VII (D<sup>2</sup> = 3171872.80) followed by Cluster I and Cluster IV (D<sup>2</sup> = 2312885.53), Cluster II and Cluster VII (D<sup>2</sup> = 2226978.39), Cluster V and Cluster VII (D<sup>2</sup> = 1568183.72), Cluster II and Cluster IV (D<sup>2</sup> = 1517203.9), Cluster III and Cluster V (D<sup>2</sup> = 85919.189) followed by Cluster IV and Cluster VII (D<sup>2</sup> = 80396.26). The range of intra Cluster values and higher inter Cluster distances than intra Cluster distances represented the high genetic diversity among genotypes

indicating the possibility to create variability using the genotypes. Mean values for all the fourteen characters were evaluated which are summarized in Table-3 below and it was seen that for days to 50% flowering mean values in Cluster IV (61.00) was lowest and the highest values was observed for Cluster I (93.12). For days to maturity Cluster I (126.17) and Cluster III (95.17) had maximum and minimum values respectively. For Leaf area Cluster V (505.6833) was highest and Cluster III (369.65) was lowest. While Cluster I (260.55) and Cluster IV (224.4889) were the maximum and minimum values respectively. Stomatal count per microscopic field were maximum for Cluster VI (20.66667) while minimum for Cluster I (16.19048) similarly Cluster I (4.089286) showed the minimum transpiration rate on the other hand Number of leaves per plant was high for Cluster I (11.95238) and least for Cluster III (8.75), Cluster V (33.56833) revealed maximum values for flag leaf length, Panicle length was found maximum for Cluster IV (24.70556), Cluster VII (3.837667) showed highest magnitude for test weight, Green fodder yield per plant was recorded high for Cluster I (447.7171) and least for Cluster II (281.8267), Cluster VII (187.8333) and Cluster IV (108.9378) showed the maximum and minimum values for dry fodder yield per plant respectively. Grain yield per plant was maximum for Cluster VI (56.96856). it was clearly observable in the given data that Cluster I was grouped with genotypes performing at par for traits *viz* green fodder yield per plant, leaf temperature, number of leaves per plant, days to 50% flowering, plant height and days to maturity. transpiration rate with value of 99.1% followed by green fodder yield per plant (0.2%), days to 50% flowering, grain yield per plant (0.1%), leaf temperature, number of leaves per plant, days to maturity plant height, dry fodder yield per plant, panicle length at maturity, test weight, leaf area, stomatal count per microscopic field and flag leaf length were contributing towards divergence. Mainly principal component analysis is pursued to minimize the large complex datasets into smaller dimension interpretable results the analysis divides the number of components extracted is equal to the number of variables being analyzed. The first component can be expected to account for a large amount of the total variance. Each succeeding component accounts for progressively smaller amounts of variance (Kavithamani *et al.*, 2019) [6] out of which Data were considered in each component with Eigen values more than 1 as per the suggestions given by Brejda *et al.* (2000) [5], that is only components with eigen values > 1 was selected in the present study, as visualized Fig 1: Scree plot clearly depicts that the first four principal components should be selected out of 14, though each principal component contributed for the divergence, maximum divergence was observed for Principal component-1 (41.6%) followed by Principal component-2 (16.6%), Principal component-3 (11.3%), Principal component-4 (7.4%) as stated in Table 4, for principal component-1 characters like green fodder yield per plant, dry fodder yield per plant, number of leaves per plant, days to 50% flowering, days to maturity, plant height and leaf temperature contributed the higher factor loadings as mentioned in Table-5, similarly for principal component-2 higher factor loadings were observed for days to 50% flowering, days to maturity, test weight and flag leaf length, for principal component-3, flag leaf length, leaf temperature and plant height, for principal component-4, panicle length, leaf area, grain yield per plant and test weight. Abraha *et al.* (2015) [8] on evaluating 25 sorghum genotypes through D<sup>2</sup> analysis and observed similar results for grain yield, biomass, stay-green and leaf area similarly Prasad and Sridhar (2019) [9] found characters like 100 seed weight. Ear length, grain yield

per plant, number of leaves per plant, days to 50% flowering and days to maturity contributing for maximum divergence in 40 yellow pericarp sorghum genotypes. Principal component analysis conducted by Prasanth *et al.* (2021) <sup>[10]</sup> exhibited the

similar results for Fresh biomass, days to flowering and plant height similarly Rohila *et al.* (2022) <sup>[11]</sup> for green fodder yield, dry fodder yield, and plant height Kavithamani *et al.* (2019) <sup>[6]</sup> for 100 seed weight and Plant height.

**Table 1:** Distribution of 30 sorghum genotypes into Clusters

S. No.	Cluster No.	No. of germplasm	Name of germplasms
1	I	14	Gird-11, Gird-21, Gird-8, Gird-31, JJ-1041, Gird-29, Gird-34, Gird-48, Gird-30, Gird-1, Gird-45, Gird-49, CSV-15, Gird-3
2	II	3	SPV-2495, JJ-938, SPV-2507
3	III	4	SPV-2357, SPV-2376, RVJ-1862, JJ-741
4	IV	3	SPV-2493, CSV-17, SPV-2375
5	V	2	SPV-2499, Gird-12
6	VI	3	SPV-2501, SPV-2506, Gird-41
7	VII	1	Gird-5

**Table 2:** Average intra and inter Cluster D2 values of 30 sorghum genotypes

Cluster	I	II	III	IV	V	VI	VII
I	1090.71	90040.827	659719.71	2312885.53	285865.119	1172239.48	3171872.8
II		2032.887	274371.16	1517203.9	60104.562	631204.99	2226978.39
III			22327.78	525486.55	85919.189	91118.1	963659.55
IV				27422.98	986442.576	208663.52	80396.26
V					5545.897	309850.01	1568183.72
VI						19041.46	499626.57
VII							0

**Table 3:** Mean values of 14 characters of 30 sorghum genotypes arranged in 7 Clusters

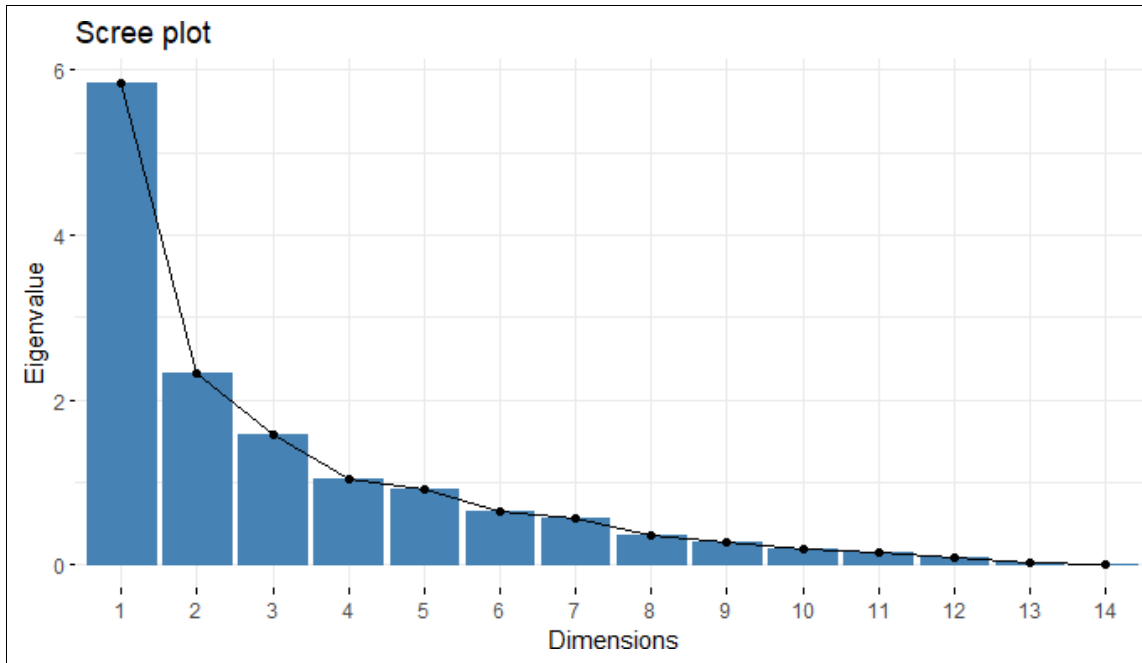
Characters	Clusters						
	I	II	III	IV	V	VI	VII
DTF	93.12	68.33	64.50	61.00	82.17	79.56	91.00
DTM	126.17	102.00	95.17	95.89	115.50	113.44	123.00
LA	475.07	441.97	369.65	452.04	505.68	490.52	451.17
PH	260.55	251.31	232.22	224.49	239.33	258.32	224.63
SCF	16.19	17.11	20	19.78	19.83	18.89	20.67
TR	4.09	8.92	17.71	28.85	13.04	22.45	34.46
LT	37.40	34.04	34.93	34.72	35.05	36.97	34.35
NLP	11.95	10.55	8.75	10.11	11.33	11.33	11.33
FLL	32.65	26.66	33.09	30.56	33.57	31.46	10.44
PL	19.62	22.28	20.14	24.70	22.76	23.03	17.67
TW	2.97	3.16	2.70	2.78	2.91	2.87	3.84
GFYP	447.72	281.82	284.30	318.37	315.39	417.48	425.60
DFYP	183.31	118.50	110.94	108.94	123.26	170.10	187.83
GYP	49.87	49.15	48.46	39.48	56.31	56.97	55.96

**Table 4:** principal component analysis of the measured fourteen characters

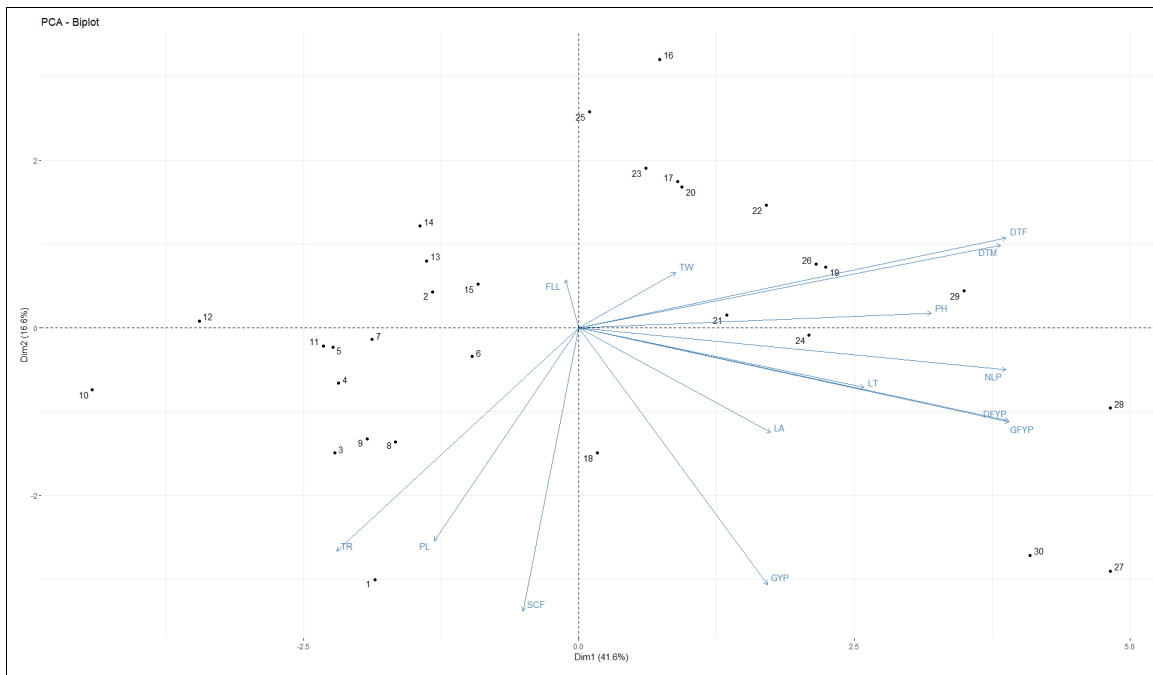
	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12	PC13	PC14
Standard deviation	2.415	1.526	1.257	1.021	0.959	0.798	0.751	0.603	0.521	0.431	0.383	0.299	0.173	0.08
proportion of variance	0.416	0.166	0.113	0.074	0.065	0.045	0.04	0.026	0.019	0.013	0.01	0.006	0.002	0.00047
Cumulative proportion	0.416	0.582	0.695	0.77	0.836	0.881	0.922	0.948	0.967	0.98	0.991	0.997	0.999	1
Eigen values	5.832	2.329	1.613	1.042	0.92	0.637	0.564	0.364	0.271	0.186	0.147	0.089	0.029	0.006

**Table 5:** factor loadings of the study characters first four principal components

	PC1	PC2	PC3	PC4
DTF	0.377	0.165	-0.064	0.007
DTM	0.372	0.152	-0.056	0.037
LA	0.169	-0.192	0.044	0.405
PH	0.311	0.027	0.117	-0.119
SCF	-0.049	-0.521	-0.071	-0.43
TR	-0.214	-0.411	-0.21	-0.213
LT	0.252	-0.11	0.293	-0.453
NLP	0.377	-0.077	-0.047	0.122
FLL	-0.012	0.087	0.624	0.137
PL	-0.128	-0.391	0.093	0.475
TW	0.085	0.101	-0.653	0.157
GFYP	0.379	-0.173	-0.06	-0.097
DFYP	0.379	-0.17	-0.052	-0.028
GYP	0.166	-0.472	0.089	0.299



**Fig 1:** screen plot of principal component analysis plotted between principal components percentage of explained variance



**Fig 2:** BIPLLOT for principal component analysis

**Conclusion**

from all the data collected and analyzed in the study it can be concluded that on the basis of  $D^2$  values varieties namely Gird-11 and Gird-21 showed least divergence while varieties Gird-1 and Gird-5 showed maximum distance between therefore hybrids between them will be a heterotic hybrid with maximum variability, higher magnitude of inter Cluster values than intra Cluster values indicated the presence of a good deal of variability within Clusters, 30 sorghum genotypes were found to be most diverse for the character; transpiration rate. On the basis of the mean values of Clusters for various characters it can be concluded that for improvement in character of green fodder yield per plant varieties namely Gird-11, Gird-21, Gird-8 can be utilized similarly for improvement in panicle length varieties like SPV-2493, CSV-17, SPV-2375 can be used, for improvement in dry fodder yield per plant, test weight,

transpiration rate and stomatal count genotype Gird-5 can be utilized or we can say that Gird-5 is useful for making a drought tolerant variety. On the basis of the principal component analysis, it was concluded that characters like green fodder yield per plant, dry fodder yield per plant, days to flowering, days to maturity, plant height, grain yield per plant, leaf temperature, test weight, leaf area, number of leaves per plant were highly diverse. And it can be stated that for these characters the following genotypes can be utilized as parents in hybridization programs.

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