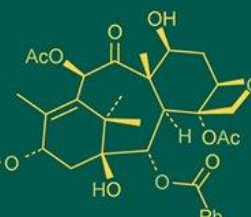
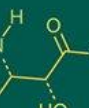
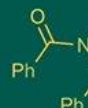
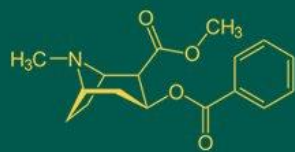


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M Mohan Krishna
Department of Genetics and
Plant Breeding, College of
Agriculture, Professor
Jayashankar Telangana
Agricultural University,
Hyderabad, Telangana, India

Dr. AV Umakanth
Principal Scientist,
Department of Genetics and
Plant Breeding, Indian
Institute of Millets Research
(IIMR), Rajendranagar,
Hyderabad, Telangana, India

Dr. CV Sameer Kumar
Senior Professor and
University Head, Department
of Genetics and Plant
Breeding, PJTAU,
Rajendranagar, Hyderabad,
India

Dr. VM Malathi
Scientist, Department of
Biochemistry, Indian Institute
of Millets Research (IIMR),
Rajendranagar, Hyderabad.,
India

Corresponding Author:
M Mohan Krishna
Department of Genetics and
Plant Breeding, College of
Agriculture, Professor
Jayashankar Telangana
Agricultural University,
Hyderabad, Telangana, India

Studies of genetic divergence and interrelationships in sweet sorghum (*Sorghum bicolor* (L.) Moench)

M Mohan Krishna, AV Umakanth, CV Sameer Kumar and VM Malathi

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Abstract

A field experiment was conducted at IIMR during kharif 2025 to study the nature and magnitude of genetic diversity and identify promising parents in sweet sorghum for biofuel-related traits like brix (%), green cane yield and related characters in 19 sweet sorghum genotypes. Depending on the D^2 values, these were grouped into 3 clusters. Cluster II was the largest, consisting of 15 genotypes and cluster III was the least with one genotype. Among the 13 characters studied, plant height contributed the most, followed by brix percent, towards the divergence of genotypes. The highest intra-cluster distance was observed for cluster II, while the maximum inter-cluster distance was observed between clusters I and II. Based on the D^2 values, the genotypes from diverse clusters would be derived and used in the hybridization program to generate a wide range of transgressive segregants for genetic enhancement of sweet sorghum for biofuel-related traits. The total variability among the genotypes was partitioned into three principal components (PC I, PC II, and PC III), which together accounted for 96.9% of the total variation. Variation relative to the first component was associated with plant height, total sugar index and ethanol yield. The second principal component was associated with days to 50% flowering and fresh stalk yield. The third principal component was associated with juice extraction and plant height.

Keywords: Sweet sorghum, principal component analysis, Mahalanobis D², genetic divergence, Tocher's clustering

Introduction

The rapid increase in the global population and economic development, especially in Asia, is boosting the demand for energy, food, and feed. Dependence on fossil fuels to satisfy this demand results in high atmospheric pollution, rapid resource depletion, and greenhouse gas emissions that contribute to global warming. From 2015 to 2024, world petroleum consumption grew from 95.8 million barrels per day to 102.8 million barrels per day, according to the U.S. Energy Information Administration (U.S.E.I.A). In India, consumption increased from 3,848 to 5,599 thousand barrels per day, representing a significant rise and releasing thousands of tonnes of greenhouse gases. The foremost challenge today is to reduce the global carbon footprint. Biofuels are especially crucial because they are the only renewable liquid fuels compatible with existing vehicles. Sweet sorghum shows significant promise as a crop for ethanol production worldwide due to its adaptability to diverse growing conditions.

Sorghum (*Sorghum bicolor* (L.) Moench), a member of the Poaceae family, is a diploid ($2n = 2x = 20$) and often cross-pollinated crop. It tolerates drought and high temperatures, making it a vital crop in semi-arid regions of Africa, Central America, and South Asia. With nearly 40 million hectares cultivated globally, it ranks as the fifth most common crop used for food, fodder, and alcohol production (Hossain *et al.*, 2022) [10].

Sweet sorghum is embedded with a sugar-rich stalk similar to sugarcane. Due to its high sugar content and ease of extraction, it is one of the leading biofuel feedstocks. Its stalk juice contains about 75% sucrose, along with 2.6% fructose and glucose. These sugars are ideal for bioethanol production because they ferment easily into ethanol (Bandara *et al.*, 2020) [1]. The plant can produce juice constituting up to 78% of its biomass, and its Brix value ranges from 14 to 23% (Vinutha *et al.*, 2014) [5]. As a C_4 plant, sweet sorghum has high photosynthetic efficiency, resulting in rapid growth, high sugar accumulation, drought resistance, and waterlogging tolerance. It is well suited to subtropical and temperate regions worldwide.

All parts of the plant have economic uses: the grain as food or feed, the stalk for fuel, and the fiber for mulch or animal feed (Sales *et al.*, 2021) ^[4]. Notably, sweet sorghum has a crop cycle of about four months, compared to sugarcane's 12-13 months, allowing three harvests per year with 300% cropping intensity, and water use is nearly three times lower (8,000 m³/ha) than sugarcane (36,000 m³/ha) (Aruna *et al.*, 2018) ^[11].

For effective breeding, selecting divergent parent plants is critical because heterosis depends on the genetic divergence between parents. Proper germplasm classification helps breeders identify genetically diverse parents for hybridization. Genetic diversity is vital for crop improvement, offering a way to quantify the relationship between geographic and genetic variation through measures such as Mahalanobis distance (Mahalanobis, 1936) ^[2]. Sorghum exhibits high genetic diversity due to its adaptability across various climates and gene flow among different races. D² statistics assist in distinguishing cultivars based on genetic differences and selecting genetically distinct parents for breeding (Oliveira *et al.*, 2020) ^[3].

Thus, assessing available accessions for genetic diversity and selecting top performers is essential. This study aims to evaluate the genetic divergence among sweet sorghum genotypes and identify promising parent plants for biofuel

traits such as brix percent, juice percent, juice yield, fresh stalk yield, and other relevant characteristics.

Materials and Methods

The material for the present study comprised 19 sweet sorghum genotypes (Table 1). The experiment was conducted at the IIMR farm (Indian Institute of Millets Research), Rajendranagar, Hyderabad, during kharif 2025. Each genotype was raised in 2 rows of 3 m length spaced at 60 cm with an interplant distance of 15 cm. The experiment was laid out in a Randomized Block Design with three replications. In each entry, five randomly selected plants were utilized to collect data on different characters viz., days to 50 percent flowering, days to maturity, plant height (cm), stem girth (cm), juice yield (l/ha), brix content (%), juice extraction (%), total soluble sugar (%), total sugar index (t/ha), grain yield (t/ha) and ethanol yield (l/ha) and the data was subjected to statistical analysis. Wilk's criterion was used to test the significance of pooled differences in mean values for all characters. Genetic diversity was studied using Mahalanobis D² and clustering of genotypes was done according to Tocher's method as per Rao (1952) ^[8], while principal component analysis was carried out as per Rao (1964) ^[9]. All statistical analyses were carried out using WINDOSTAT version 9.1 software.

Table 1: Sweet sorghum genotypes used in the study

S. No.	Genotypes	Pedigree	S. No.	Genotypes	Pedigree
1	RSSV 260	Selection from IS 5353	11	370B	27A × (90001B × NSSB 1005)-4-1-2
2	11 NRL	(RSCN 2103 × SSV 84)-1-3-2-1	12	15B	AKMS14B × IS25017-1
3	SPV 2074	Selection from SSV 74	13	19B	AKMS14B×IS25017
4	St11B ₂	[(ICSV 4 tall × Bulk Y) D 181]-1-1-1	14	51B	463B×332B
5	473B	463B×382B	15	55B	B POP F 2004
6	PMS71B	Selection from ICSB 13019 × (Gidda Maldandadi × 296B)	16	75B	F122B
7	456B	(91B × 1B) × CS 3541	17	91B	296B×IS18551
8	335B	27A × (NSSB 13 × ICSB 699)-1-1-1	18	95B	463B×B58586
9	337B	27A × (NSSB 1002 × NSSB 1005)-5-1-2	19	97B	463B×332B
10	363B	27A × (ICSB 687 × ICSB 702)-9-1-3			

Results and Discussion

The analysis of variance using a randomized block design indicated significant variation for all 13 traits investigated, indicating the presence of a substantial level of genetic diversity among the sweet sorghum accessions.

Principal component analysis

The total variability among the genotypes was partitioned into three principal components, namely PC I, PC II and PC III, which together accounted for 96.9 percent of the total variation (Table 2) (fig 1).

The first principal component explained 90.06 percent of the total variability. The second and third principal components explained 4.95 and 1.97 percent of the total variability, respectively. From the table, it is clear that the first principal factor showed high positive loading for plant height (0.813), total sugar index (0.318), and ethanol yield (0.148), whereas it showed high negative loading for grain yield (-0.337), total soluble solids (-0.166). Principal factor two enabled high positive loading for days to flowering (0.519), fresh stalk yield (0.466), whereas it showed high negative loading for total sugar index (-0.202), total soluble solids (-0.155). Principal factor three showed high positive loading for juice extraction (0.305), and plant height (0.300). Elenen *et al.*

(2019) ^[15] in a similar study for 13 characters in sweet sorghum germplasm, observed 96.90% of the total variation to be accounted for by the first seven principal components.

Table 2: Principal component analysis for yield and yield component traits in sweet sorghum genotypes

Metric	1 Vector	2 Vector	3 Vector
Eigen Value (Root)	2475.72000	136.18220	54.32203
% Var. Exp.	90.06024	4.95395	1.97609
Cum. Var. Exp.	90.06024	95.01418	96.99028
1 Days to 50% flowering	0.08826	0.51971	0.27589
2 Days to maturity	0.00000	0.00000	0.00000
3 Plant height (cm)	0.81368	0.08164	0.30002
4 Stem girth (cm)	-0.14832	0.29796	0.09227
5 Total biomass yield (t/ha)	-0.05626	0.36150	-0.16263
6 Fresh stalk yield (t/ha)	-0.07474	0.46665	0.00650
7 Juice yield (l/ha)	0.14573	0.21628	-0.12494
8 Brix (%)	0.04542	0.39381	-0.57986
9 Juice extraction (%)	0.10227	0.06174	0.30511
10 Total soluble solids (%)	-0.16698	-0.15508	0.21084
11 Total sugar index (t/ha)	0.31837	-0.20281	-0.41433
12 Grain yield (t/ha)	-0.33799	0.03684	0.08660
13 Ethanol yield (l/ha)	0.14882	-0.11745	-0.36098

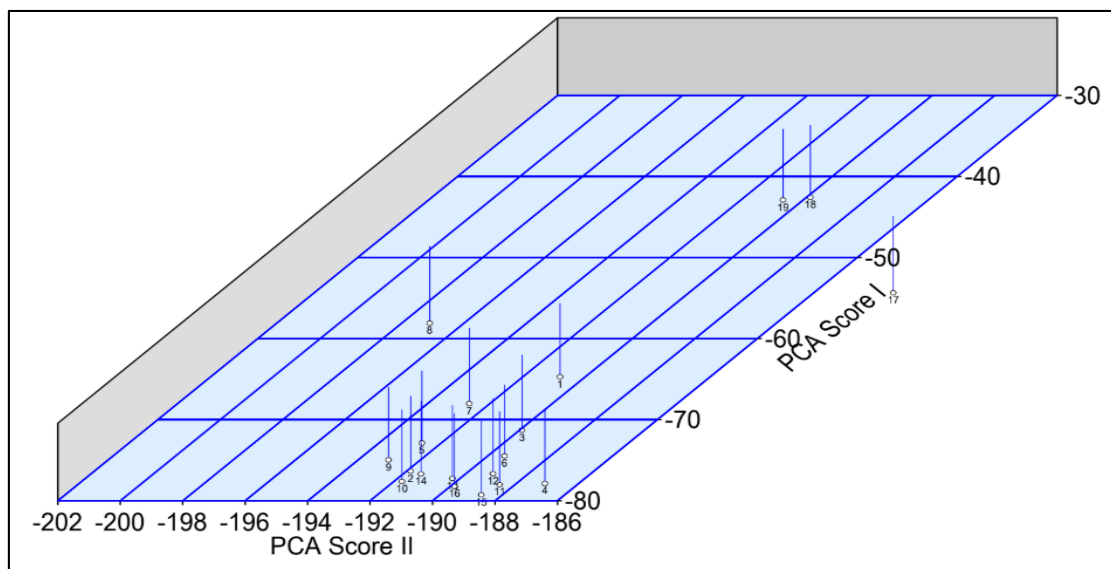


Fig 1: Principal component analysis diagram for sweet sorghum genotypes

Cluster Divergence

The 19 genotypes were grouped into 3 clusters (fig 2) based on D^2 values such that the genotypes belonging to the same cluster had an average D^2 value smaller than those belonging to different clusters. The cluster analysis led to the formation of three clusters. Cluster I had the maximum number of genotypes, i.e., 15 followed by Cluster II (3) and Cluster III is monogenotypic, indicating the uniqueness of the genotype included in this cluster when compared to other genotypes included in the study. Intra and inter-cluster distances were calculated for different clusters as described in Table 4. The intra-cluster distances were lower than the inter-cluster distances. Sameer Kumar *et al.* (2010) in a genetic diversity study involving 60 sorghum genotypes observed that the inter-cluster distance in most cases was higher than the intra-cluster distance, indicating wider genetic diversity between the genotypes of different clusters. Intra-cluster distance was maximum in cluster II (145.09) followed by cluster I (53.26) and cluster III (0.00). The genotypes placed within the cluster were less divergent.

Inter-cluster distance (Table 4) was maximum between cluster I and cluster II (881.19), followed by cluster I and cluster III (382.17), indicating wide genetic divergence among these clusters. These findings are in conformity with the findings of Prasad and Biradar (2017). Inter-cluster distance was found to be minimum between cluster II and cluster III (272.32), indicating close genetic similarity between these clusters.

The crosses between genotypes within the same cluster are unlikely to result in transgressive segregants since they show little divergence. To increase the likelihood of producing desired transgressive segregants, hybridization programs should give priority to crossing parents from various clusters with the highest genetic difference. It is advised to choose genotypes from widely dispersed clusters for hybridization since intercrossing these divergent groupings expands the population's genetic basis. This raises the likelihood of crossing over, which can disrupt closely coupled genes and reveal hidden genetic variety.

Cluster Mean for Different Characters

Cluster-wise mean of 13 trait was calculated and presented in Table 5. Cluster II showed high mean for traits viz. days

to flowering (91.22), days to maturity (131.22), plant height (336.33), stem girth (1.94), total biomass yield (42.28), fresh stalk yield (34.18), brix percent (17.48), total soluble solids (15.42), total sugar index (2.16) and ethanol yield (1149.69). Cluster III showed high mean for juice extraction (56.83) whereas for trait grain yield cluster I and III showed high mean.

The lowest means for days to flowering and days to maturity was observed in cluster III indicating that the genotypes grouped in this cluster are early flowering and early maturing in nature. Similar findings were also reported by Patankar *et al.* (2005) [12]. The clusters II have recorded high mean performance for various characters. Therefore, in hybridization, the genotypes chosen from these clusters can serve as parents. Ethanol output and other characteristics in sweet sorghum were shown to vary widely based on cluster means. In order to create high-yielding hybrids or varieties, it is proposed that lines from the most diversified clusters be used as parents in hybridization programs. Additionally, it has been noted that parents with great genetic variety and high yields may produce the most productive hybrids. Similar type of results were given by Swamy *et al.* (2018) [6].

Percent Contribution of Different Traits to Genetic Divergence:

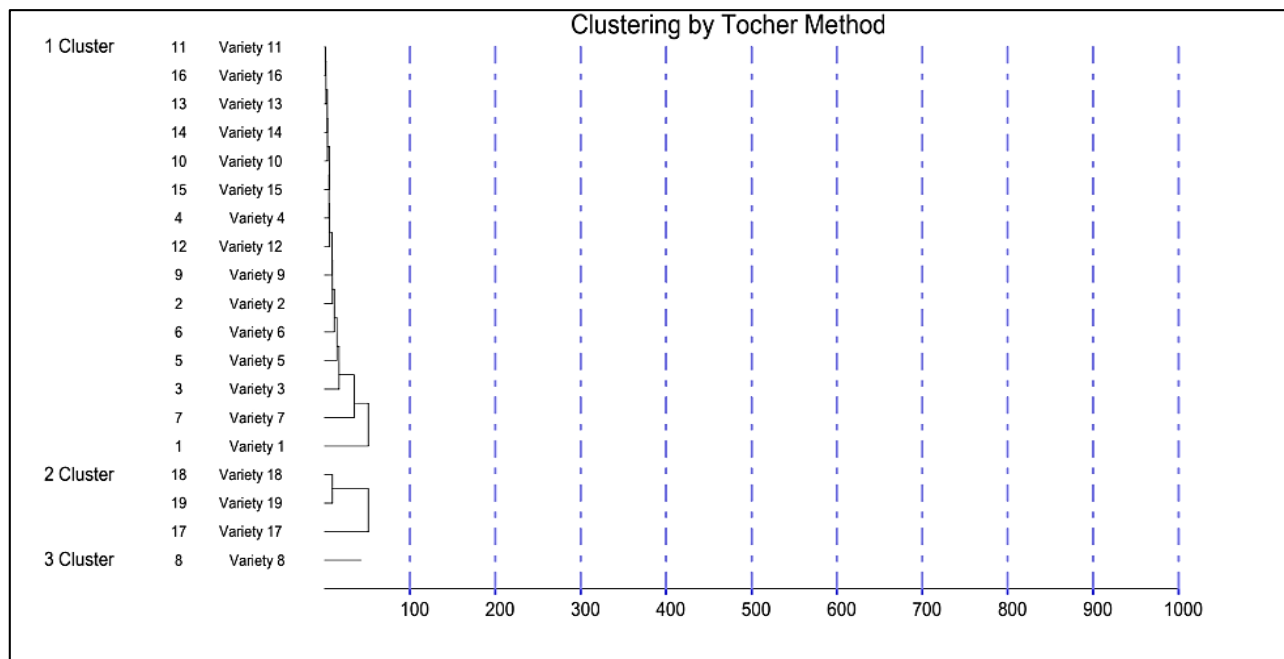
The number of times that each of 13 characters appeared in first rank and its respective percentage contribution towards genetic divergence is presented in Table 3 and fig 4. The results showed that, plant height contributed maximum to genetic divergence (66.67%) by obtaining 114 times first rank, followed by brix (8.19%) by 14 times, juice extraction (5.85%) by 10 times, stem girth (5.85%) by 10 times and days to 50% flowering (5.26%), indicating their predominant role in differentiating the genotypes. Similar results were reported by Elangovan *et al.* (2014) [13].

Out of 13 traits examined in the study fresh stalk yield and total soluble solids contribute the least towards divergence. It accounts for 0.58% of the contribution and ranked first 1 time.

Breeders must comprehend trait-wise contribution in order to concentrate selection efforts on traits that provide the greatest genetic variety.

Table 3: Percent contribution of different traits

S. No	Source	Times Ranked 1st	Contribution%
1	Days to 50% flowering	9	5.26
2	Days to maturity	-	0
3	Plant height (cm)	114	66.67
4	Stem girth (cm)	10	5.85
5	Total biomass yield (t/ha)	-	0
6	Fresh stalk yield (t/ha)	1	0.58
7	Juice yield (l/ha)	-	0
8	Brix (%)	14	8.19
9	Juice extraction (%)	10	5.85
10	Total soluble solids (%)	1	0.58
11	Total sugar index (t/ha)	2	1.17
12	Grain yield (t/ha)	7	4.09
13	Ethanol yield (l/ha)	3	1.75

**Fig 2:** Dendrogram showing clustering of sweet sorghum genotypes**Table 4:** Intra (bold) and inter-cluster average distances among clusters of sweet sorghum genotypes

Cluster	Cluster 1	Cluster 2	Cluster 3
Cluster 1	53.26	881.19	382.17
Cluster 2		145.09	272.32
Cluster 3			0.00

Table 5: Cluster mean values: Tochers method

	DFF	Days to maturity	Plant height	Stem girth	Total biomass Yield (t/ha)	Fresh stalk yield (t/ha)	Juice yield (l/ha)	Brix (%)	Juice extraction (%)	Total soluble Solids (%)	Total sugar index (t/ha)	Grain yield (t/ha)	Ethanol yield (t/ha)
Cluster I	74.80	114.80	152.27	1.50	12.30	9.59	4650.85	12.22	47.42	10.82	0.52	1.23	275.38
Cluster II	91.22	131.22	336.33	1.94	42.28	34.18	14005.00	17.48	52.92	15.42	2.16	1.11	1149.69
ClusterIII	71.00	111.00	265.67	1.33	14.07	10.53	5300.00	11.30	56.83	10.02	0.62	1.23	291.13

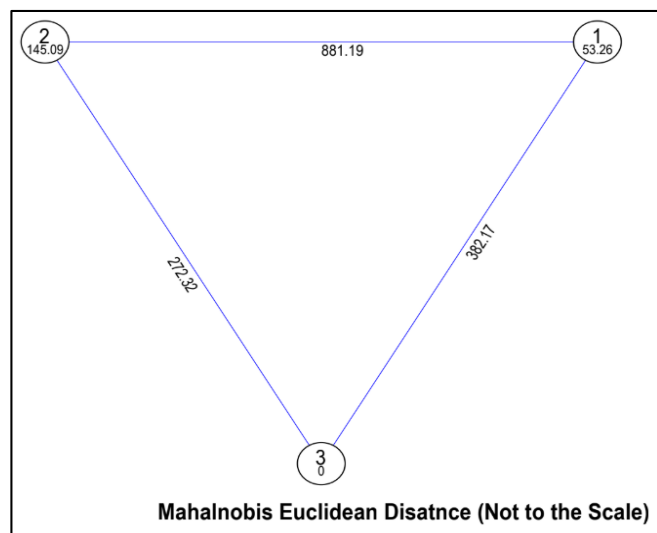
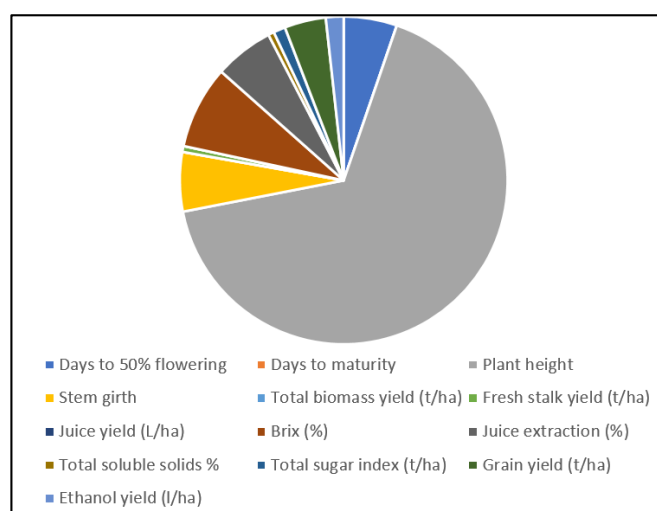


Fig 3: Mahalanobis Euclidean distances



Conclusion

Genetically varied and agronomically superior genotypes were chosen using data from the Mahalanobis D^2 study of genotypes. The genotypes that were especially good in one or more attributes appeared to be desired. Inter-crossing divergent groups would increase the likelihood of crossing over, which would break the unwanted bond and reveal latent potential variability. The inter-cluster distance between clusters I and II in the current study was at its highest, showing significant genetic divergence between these clusters. It is anticipated that the offspring of such varied crossings will exhibit a broad range of genetic diversity, increasing the possibility of isolating transgressive segregants in later generations. The majority of the yield and biofuel-related traits, such as plant height, biomass yield, fresh stalk yield, brix, total sugar index, and ethanol yield, performed better in cluster II, according to cluster mean analysis. This suggests that genotypes from this cluster can be used as promising parents. Cluster III, on the other hand, showed increased juice extraction and early flowering and maturity, suggesting its value in breeding for genotypes with early and effective juice recovery. Characteristics such as plant height, total sugar index, ethanol yield, days to 50% blooming, fresh stalk yield, juice extraction, and brix (%) were found to be extremely different based on the principal component analysis. Furthermore, it can be deduced that

genotypes that perform better for these qualities could be used as parents in hybridization programs to produce high-yielding and biofuel-efficient genotypes.

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