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Research Note

Identification of diverse forage sorghum genotypes based on cluster analysis

N. Y. Akshitha*, Pummy Kumari, S. K. Pahuja, Neeraj Kharor, Amit and D. S. Phogat

Forage Section, Dept. of Genetics and Plant Breeding, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana-125004
***E-Mail**: akshithany@gmail.com

Abstract
A field experiment comprising of 50 genotypes of forage sorghum was laid out in CRBD. Enough variability was observed among the genotypes under study as revealed by ANOVA. Genetic divergence analysis grouped the genotypes into six clusters based on quantitative and qualitative traits. Cluster I had the maximum number of genotypes, followed by clusters III and V with ten genotypes each; cluster IV had nine genotypes; cluster II had seven genotypes and cluster VI had only one genotype. The maximum intra cluster distance was observed in clusters I followed by cluster V and cluster III. Intra-cluster distance is measure of the amount of variability present within cluster. The highest inter-cluster distance was found between the clusters I and III followed by the clusters I and V which confirms that genotypes in these clusters are more diverse. Use of genetically diverse genotypes of clusters VI and III in hybridization program would help to evolve the genotypes with high fodder yield and good quality.

Keywords: Forage sorghum, diversity, green fodder, quality

Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth most important cereal crop in the world. It is mainly cultivated as fodder crop in arid and semi-arid regions of the country (Elangovan and Babu, 2015) with approximately 54 per cent of the total cultivated fodder area during *Summer* and *Kharif* season (Dagar, 2017). The most cost-effective feed for milch and draught animals is green fodder. Forage sorghum is presumed to contribute 20–45 percent dry weight of the total feed during the *Kharif* season and around 60 per cent in the lean periods of summer and winter for dairy animals (Sorghum vision, 2030).

Despite being the world's foremost producer of milk, India faces a challenge such as poor milk outturn of 1538 kg per animal, which falls well below the global average of 2238 kg. This accounts for less per capita availability and significantly contributes to the issue of malnutrition. Poor milk output is due to severe shortage of animal feed (Vijay *et al*., 2018). Country faces shortage of various forms of animal feeds as follows: concentrate feed materials (44 percent), dry fodder (10.95 percent), and green fodder (35.6 percent) throughout the nation (IGFRI Vision, 2050). The demand for green and dry fodder demand will rise to 1012 million tonnes and 631 million tonnes, respectively by the year 2050. Whereas with the current rate of forage supply expansion, there will be 18.4% and 13.2% deficit in green and dry fodder by the year 2050 in India. To bridge the demand and supply gap, the amount of green fodder production must increase by 1.69 percent annually, but the country's area under fodder crops is only 4% of total arable land (8.4 million ha) and has hardly increased in recent years (Halli *et al*., 2018). Thus forage breeders should focus on development of high green and dry fodder yielding forage crop varieties that could yield more biomass per unit area (Subbulakshmi *et al.,* 2023).

Knowledge on genetic architecture of quantitative traits helps the breeders in choosing desirable parents for breeding program. To produce superior genotypes with resistance against abiotic and biotic stresses more diverse genotypes should be involved in crossing program. Available genetic diversity in any crop will facilitate further improvement of crop. In conventional plant breeding approaches, morphological markers are major tools to study the genetic variability (Ahlawat *et al.,* 2018). Morphological marker study is easy and cost-effective technique. These easily observable morphological markers are very useful in preliminary evaluation. Various biometrical techniques like correlation, path coefficient analysis, principal component analysis and D^2 technique has been used to estimate the genetic variability in all the crops. Cluster analysis helps to categorize and group similar genotypes based on their characteristics. By employing D^2 or cluster analysis, researchers can identify patterns, similarities, and differences among genotypes, aiding in the understanding of genetic diversity and facilitating informed decision-making in areas such as breeding, conservation, and crop improvement. Over the years, various studies were conducted to estimate the extent of genetic diversity in cultivated sorghum specially focused on grain sorghum. But in the present experiment we have studied the extent of genetic diversity based on fodder yield, quality and seed traits and grouped them in various clusters.

Fifty forage sorghum genotypes were grown in CBRD (Complete Randomized Block Design) with three replications during the *Kharif* 2021. Crop was grown in Research Area of Forage Section, Department of Genetics and Plant Breeding, Chaudary Charan Singh Haryana Agricultural University, Hisar, India which is situated in semi-arid tropics. Each genotype was sown in two rows spaced at 30 cm with a spacing of 10 cm between plants. All the recommended agronomic practices were followed. Data was recorded on individual plant basis on five randomly selected plants from each line for three qualitative, 18 quantitative and four biochemical characters. Leaf midrib colour of 5^{th} fully opened leaf was recorded as yellow green and white, most of the morphological parameters were recorded at the stage of 50% flowering, ear head compactness and shape were recorded at physiological maturity. Traits such as seed germination percent, seedling dry weight, seedling length, seed vigour index I and II were recorded from germinated seed. Green and dry fodder yield per plant per day was also calculated by dividing green and dry fodder yield per plant by number of days of harvest which helps to identify genotypes suitable for fodder purpose having vigorous growth. Hydrocyanic acid content (HCN) was estimated from fresh young plants (30 days after sowing) as per method given by Gilchrist *et al.* (1967). Total Soluble Solids (TSS) content was recorded using refractometer. For the estimation of dry fodder yield a 500 gm sample of green fodder was taken at the time of 50% flowering and after drying the samples were ground and used for estimation of crude protein (%) by Micro-Kjeldhal's method. Morphological diversity analysis of all the twenty-two characters under study was carried out on the basis of their mean values. Analysis of variance (ANOVA) was carried out using model given by the Panse and Sukhatme (1969) using OP-STAT software. D^2 statistics (Mahalanobis, 1936) was used for estimation of genetic diversity among genotypes using the Ward's method as described by Rao (1952) by employing INDOSTAT software.

Fifty genotypes were grouped into different classes based on qualitative traits as furnished in **Table 1**. It was observed that 37 genotypes were having yellow green midrib, and 13 genotypes were having white midrib colour. Panicle density at maturity grouped the genotypes into very loose (two genotypes), loose (seven genotypes), semi loose (sixteen genotypes), semi compact (twenty-two genotypes) and compact (three genotypes) types. Nine genotypes had panicle broader in lower part, one genotype had panicle broader in upper part, eighteen genotypes were pyramidal in their panicle shape and twenty-one genotypes had symmetrical panicle.

ANOVA revealed highly significant differences for all the quantitative traits under study among the genotypes. This may be due to diverse sources of genotypes collected as well as environmental effects. D^2 statistics groups the genotypes into six clusters (**Table 2**).

Out of the six clusters, Cluster I was the largest cluster with a total of 13 genotypes followed by Cluster-III and Cluster-V with ten genotypes each. Clusters IV, II and VI have 9, 7 and one genotype respectively. Average intra and inter cluster distance values are presented in **Table 3**. The maximum intra cluster distance was observed for cluster I (857.4) followed by cluster V (750.5) and cluster III (736.2). Intra-cluster distance tells about the amount of variability present within different clusters. The highest inter-cluster distance was recorded between clusters I and III (1491.8) followed by clusters I and V (1245.4) and clusters I and II (1092.9) which indicates the existence of wider genetic diversity among the genotypes of these clusters and genetic makeup of these cluster is markedly different from that of the other cluster. Therefore, hybridization between genotypes of these clusters might lead to development hybrids with high recombination and heterosis. Clusters II and VI (430.8) had minimum inter cluster distance which indicates that the genotypes of these clusters are less diverse and closely related to each other. Clustering of the genotypes based on cluster means for various quantitative and qualitative traits helps to identify suitable forage sorghum genotypes with an appropriate trait of interest.

Mean performance of different clusters for various traits revealed wide range of differences among clusters with respect to these traits as shown in **table 4**. The average cluster means for different characters showed that cluster

Table 1. Classification of forage sorghum genotypes based on qualitative traits

Leaf mid rib colour	
Yellow green	IS 1283, IS 33844, ICSR 113, YPS 5, IS 25699, CSM 335, IS 16382, IS 21645, PFR 3, ICSR 17005, S-722, GP-2, IS 896, S-71, SSG-233, IS 12135, IS 14278, IS 2351, IS 23992, S 537, IS 29687, IS 29614, S 536, IC 485151, SPV 2312, SPV 2394, UTMC 1539, SPV 2389, SH 1488, IC 285850, IC484464, IS 33998, SSG 59-3, G 46, PGN 56, IS 34638, HJ 541
White	ICSR 90008, ICSR 93012, ICSR 93023, IS 7173, ICSR 17004, Duggi, SOR 6507, IS 5049, SPV 2314, IS 3260, IC 285913, IS 30508, IS 31681
Panicle density at maturity	
Very loose	IS 14278, SSG 59-3
Loose	ICSR 90008, IS 25699, SSG-233, IS 12135, IC484464, IC 285850, IS 30508
Semi loose	YPS 5, ICSR 93023, PFR 3, Duggi, S-71, IS 5049, SPV 2314, IS 29687, IS 29614, IC485151, IS 3260, SPV 2389, IS 33998, IC 285913, IS 34638, SH 1488
Semi compact	IS 1283, ICSR 113, ICSR 93012, IS 7173, CSM 335, IS 21645, ICSR 17004, ICSR 17005, S-722, GP-2, IS 896, IS 2351, S 537, S 536, SPV 2312, SPV 2394, UTMC 1539, G 46, PGN 56, IS 31681, HJ 541, SOR 6507
Panicle shape	
Panicle broader in lower part	ICSR 113, IS 7173, ICSR 17004, ICSR 17005, GP-2, SOR 6507, IS 896, S 537, S 536
Panicle broader in upper part	S-71
Pyramidal	ICSR 90008, YPS 5, IS 25699, CSM 335, PFR 3, Duggi, SPV 2314, IS 14278, SSG-233, IS 29687, IS 29614, IC484464, IC485151, IS 3260, IC 285850, SSG 59-3, IC 285913, IS 30508
Symmetrical	IS 1283, IS 33844, ICSR 93012, ICSR 93023, IS 21645, S-722, IS 5049, IS 12135, IS 2351, IS 23992, SPV 2312, SPV 2394, UTMC 1539, SPV 2389, SH 1488, IS 33998, G 46, PGN 56, IS 34638, IS 31681, HJ 541

Table 2. Distribution of forage sorghum genotypes in different clusters

Clusters	No. of Genotypes	Name of Genotypes
I	13	IS 1283, IS 33844, ICSR 90008, ICSR 113, YPS 5, ICSR 93012, IS 25699, CSM 335, IS 21645, PFR 3, ICSR 17004, ICSR 17005, IS 34638
II	7	ICSR 93023, SSG-233, IS 5049, SPV 2314, IS 23992, IS 29687, S 536
III	10	IS 7173, IS 16382, Duggi, GP-2, IS 896, IS 3260, UTMC 1539, G 46, IS 30508, IS 31681
IV	9	S-722, IS 2351, IS 29614, IC484464, IC485151, SPV 2312, SPV 2394, IS 33998, IC 285913
V	10	SOR 6507, S-71, IS 12135, IS 14278, S 537, SPV 2389, SH 1488, IC 285850, SSG 59-3, PGN 56
VI	1	HJ 541

Table 3. Average intra (diagonal) and inter (off the diagonal) cluster distances

Clusters	I	II	III	IV	V	VI
I	**857.4**	1092.9	1491.8	1068.6	1245.4	1195.9
II		**387.1**	782.4	759.7	763.8	430.8
III			**736.2**	1082.3	883.1	584.5
IV				**711.3**	931.4	761.3
V					**750.5**	541.7
VI						**0.0**

VI has highest mean for plant height (284.9), leaf length (86.6), leaf breadth (7.83), stem diameter (2.63), green fodder yield (369.33), and dry matter yield (89.00) with low mean for HCN content (61.71). Low value of HCN content is desirable as it is a toxic compound. Cluster VI contains single genotype (HJ 541) having higher mean values for green fodder and dry matter yield with low HCN content. So it could be used in breeding programme for fodder yield and quality improvement. Genotypes in Cluster I cannot be used directly in crossing programme because

Table 4. Mean values of different traits of forage sorghum as per cluster position

Traits	Cluster					
	I	II	III	IV	V	VI
DF	68.18	74.71	66.30	69.81	74.80	79.00
PH	162.42	221.21	214.80	166.00	205.90	284.90
NTP	1.82	2.24	1.83	2.00	1.87	1.33
NLP	19.95	24.38	19.67	19.04	18.93	15.67
LL	78.05	69.79	74.23	76.31	79.87	86.60
LB	5.69	5.59	6.25	6.16	6.28	7.83
SD	1.73	1.79	1.73	1.81	1.67	2.63
LSR	0.30	0.37	0.32	0.36	0.41	0.34
GFY	228.36	332.10	324.13	231.26	298.63	369.33
DMY	56.47	82.76	82.37	59.03	74.63	89.00
GFYPD	3.62	4.47	4.49	3.24	4.11	4.68
DMYPD	0.90	1.04	1.11	0.88	0.99	1.13
GY	43.51	45.52	46.10	38.66	47.76	45.00
HCN	77.55	75.39	77.66	84.62	73.24	61.71
TSS	6.20	9.91	5.59	9.48	7.47	7.63
CPP	7.33	7.50	7.25	6.58	6.59	5.87
CPY	4.16	6.20	5.97	3.88	4.89	5.20
SG	81.23	84.19	85.67	86.70	82.50	84.33
SL	26.46	30.76	31.04	29.81	29.28	32.77
SDW	9.01	10.45	10.47	9.98	9.79	10.92
SV-I	2265.79	2597.44	2594.68	2515.95	2457.71	2772.30
SV-II	755.26	865.81	864.90	838.65	819.24	924.10

DF: Days to 50 % flowering; PH: Plant height (cm); NTP: Number of tillers per plant; NLP: Number of leaves per plant; LL: Leaf length (cm); LB: Leaf breadth (cm); SD: Stem diameter (cm); LSR: Leaf: Stem ratio; GFY: Green fodder yield (g/plant); DMY: Dry matter yield(g/plant); GFYPD: Green fodder yield per plant per day (g/plant/day); DMYPD: Dry matter yield per plant per day (g/plant/day); GY: Grain yield(g/plant); HCN: HCN content (micro g/g); TSS: Total soluble solids (^{0} brix); CPP: Crude protein (%); CPY: Crude protein yield (g/plant); SG: Seed germination (%); SL: Seedling length (cm); SDW: Seedling dry weight (mg); SV-I: Seed vigour index-I; SV-II: Seed vigour index-II

they were inferior in terms of green and dry fodder yield. Cluster II contain genotypes that was superior in terms of number of tillers/plant (2.24), number of leaves per plant (24.38), TSS content (9.91), crude protein percent (7.5) and crude protein yield (6.2). Genotypes in cluster II can be used as parent for production of hybrids having more number of tillers with high crude protein and TSS content. The cluster III comprised of genotypes having mean values more or less similar to that of cluster II in terms of plant height, green fodder yield, dry matter yield, green fodder yield/plant/day, dry matter yield/plant/day and crude protein percent. These genotypes could be used in breeding programme for improvement of respective traits. But for improvement of fodder quality more biochemical traits need to be studied which were not included in present investigation. Genotypes in cluster IV cannot be used for quality improvement directly since it has maximum cluster mean value for HCN content (84.62). A dendrogram makes it simple to comprehend the degree of genetic divergence through graphical depiction. In a dendrogram, genotypes that fall into the same cluster are less varied than those that do not as depicted in **Fig. 1**. Findings of present investigation are in close confirmation with findings of Ahalawat *et al*. (2018), Meena *et al.* (2016), Singh *et al*. (2008), Doijad *et al.* (2016), Chikuta *et al.* (2015), Mahajan and Wadikar (2012), Prasanth *et al.* (2021) and Subbulakshmi *et al.* (2023).

It is concluded that genotypes from clusters VI and III *viz*., UTMC 1539, Duggi, HJ 541, GP-2, IS 896, G 46, IS 30508, IS 31681 could be used to obtain good recombinants for plant height, leaf length, leaf breadth, stem diameter, high green and dry fodder yield, low HCN content. The genotypes SSG-233, ICSR 93023, IS 5049, SPV 2314, IS 23992, IS 29687, S 536 from cluster II could be exploited for development of forage sorghum genotypes having more number of tillers/plant, leaves/plant, high TSS content, high crude protein percent and crude protein yield traits.

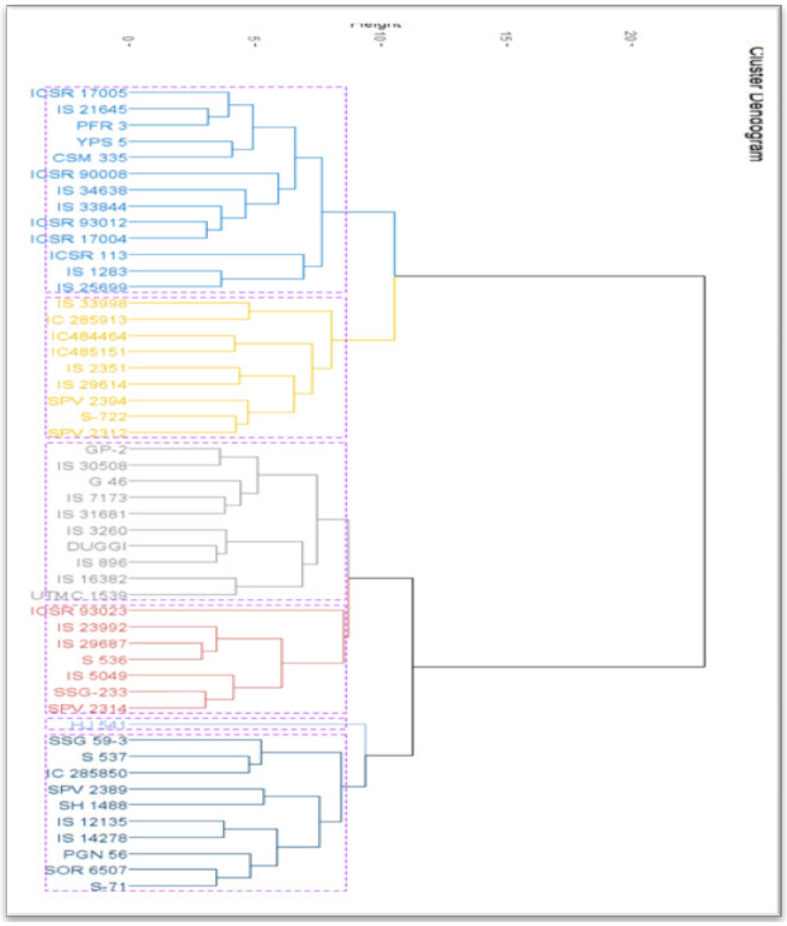

Fig.1. Dendrogram showing the clustering pattern of different forage sorghum genotypes

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