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Genetic diversity analysis by D^2 clustering of fodder yield and its related traits in forage sorghum

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ABSTRACT: The present study was undertaken during the *Kharif* season of 2017, using 73 diverse indigenous genotypes of forage sorghum. The observations were recorded for 10 morphological and eight quality parameters to assess the genetic diversity. Analysis of variance revealed sufficient variability for all the traits under study. The Mahalanobis D^2 analysis was carried out for estimation of divergence between genotypes and Tocher method was used for grouping of genotypes into different clusters. Genotypes were grouped into seven clusters. Cluster I had the maximum number of genotypes *i.e.,* 45 followed by cluster II (15) and Cluster IV (9). Cluster III, V, VI, VII had only single genotype each. Inter cluster distance was observed maximum between cluster IV and cluster VII.Cluster means for the traits under investigation showed that the genotypes in first cluster are high yielding, where genotypes IC 436522 and IC 436598 present in cluster VI and VII respectively are good for quality traits and can be further used for enhancement of yield and quality of forage sorghum.

Key words: Diversity analysis, D^2 analysis, forage sorghum

India being the largest producer of livestock support 20 per cent of the livestock population of the world, with 2.3 per cent of total geographical area only. Livestock sector contribute 4.11 per cent of total GDP and 25.6 per cent of total agriculture GDP in India. Livestock provides draught power, rural transport, manure, fuel, milk and meat. In India, hardly 5 per cent of the total cultivated area is utilized to grow fodder. Due to uncertainty of weather conditions and poor productivity, India faces acute shortage of good quality green fodder (Kour and Pradhan, 2016). Therefore, there is a need to develop improved high yielding varieties of forage crops with good nutritious value and wider adaptability.

Among forage crops, sorghum is an important fodder crop grown widely all around the world. Sorghum (*Sorghum bicolor* (L.) Moench) belongs to the genus Sorghum, tribe Andropogoneae, of the Poaceae family. India contributes 9.45 per cent of the world's sorghum production with 4.9-million-hectare area and 4.8 million tonnes of total production (FAO, 2018). In Haryana, sorghum covers 48,000-hectare area with average grain yield of 519 Kg/ha and annual grain production of 25,000 tonnes (Anonymous, 2017). Forage sorghum is very popular among the farmers of arid and semi-arid tropics areas because of its wide adaptation, rapid growth, high fodder yield, better palatability and tolerance to drought conditions. Sorghum require approximately 40-50 per cent less water than corn to produce the same amount of dry matter (Miller and Stroup, 2004). Nutritionally, sorghum fodder is excellent with potential of high digestibility and good amount of starch, crude protein, sugars and minerals like calcium, phosphorous, iron, manganese and zinc. There are some potential constrains to use sorghum as fodder because it contains compounds like hydrogen cyanide and nitrates/nitrites, that may be toxic for animals if they are ingested at a high level.

Diversity in plant genetic resources is prerequisite for crop improvement and provide opportunity for plant breeders to develop new improved cultivars with desirable characteristics. Now a day's collection, conservation and evaluation of germplasm become integral component of crop improvement program. Evaluation of large germplasm for use in crop improvement program is very laborious and source demanding task. Plant breeders are interested to evaluate genetic diversity based on morphological traits because they are inexpensive, rapid and simple to score. The agro-morphological traits are used as a powerful tool in the classification and grouping of lines, to study taxonomic status, identification, determination of genetic variation and correlation of characters.

To estimate magnitude of diversity on basis of agro-morphological traits we need various estimation techniques

such as multivariate analysis approaches, like analysis of variance and covariance, cluster analysis and principle component analysis. Multivariate data analysis provides a graphic display of the multiple traits and genotypes in way that can help in easy data interpretation. The Mahalanobis D^2 statistic helps in estimation of relative genetic divergence between genotypes and classify them into homogenous groups or clusters. The genotypes in the same cluster have little divergence while diversity between genotypes of two different clusters is usually high. Thus, representative genotypes from diverse clusters can be utilized in hybridization program depending upon breeding objective.

MATERIALS AND METHODS

The experiment comprising 73 genetically diverse genotypes of forage sorghum was conducted in randomized complete block design (RBD) with 3 replications during *Kharif* season of 2017 at research area of Forage Section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar. Hisar is situated in semi-arid sub-tropical region at 290-100N latitude and 750-460E longitude with elevation of 215.52 meter above mean sea level. The annual rainfall is around 429 mm and most of rain is received during monsoon season. Each genotype was grown in two rows of two-meter length with 30 cm spacing between rows and 15 cm spacing between plants. Normal agronomic practices recommended to the region were followed timely. Five plants were randomly selected from each plot for recording the data on traits namely, days to 50 per cent flowering, number of tillers per plant, leaf length (cm), leaf breadth (cm), stem diameter (cm), leaf: stem ratio, green fodder yield (kg/plot) and dry fodder yield (kg/plot). The data for all the above traits were recorded at the time of 50% flowering. HCN (mg/kg fresh weight) was estimated from the young shoots after 30 days of sowing by method suggested by Gilchrist *et al.* (1967). Refractometer was used to check TSS (°Brix) content. Half kilogram samples were taken from each genotype during 50% flowering and dried in field and then in the oven. After drying samples were grinded and was used to estimate quality parameters namely, crude protein content, IVDMD and minerals (Zinc, Copper, Manganese and Iron). Crude Protein (%) was estimated by Micro-Kjeldhal's method. IVDMD was assessed using method given by Tilley and Terry (1963). Atomic Absorption Spectrophotometer (AAS) was used for the estimation of zinc, iron, copper and manganese content (µg/g DM).

The mean values over replications were subjected for statistical analysis. Analysis of variance (ANOVA) for the observations recorded on different characteristics was carried out as per the standard procedure suggested by Panse and Sukhatme, (1995). Range for each character was worked out by depicting the lowest and highest values. The data collected on different characters was analyzed using 'Mahalanobis' D^2 analysis to determine the genetic divergence among the genotypes. D^2 values for all combinations of each genotype were calculated as described by Singh and Chaudhary (1977). The grouping of genotypes into different clusters was done using the Tocher's method as described by Rao (1952). Dendrogram was prepared using Indostat software.

RESULTS AND DISCUSSION

Analysis of variance revealed high significant differences among genotypes for all the characters studied and sufficient range of variability was observed for all the traits as given in Table 1. Adequate amount of variability among germplasm suggest that improvement in all these traits can be achieved by using appropriate selection strategies. The high amount of genetic variability for many of these traits has been earlier reported by Ahalawat *et al*. (2018) and Khandelwal *et al.* (2016).

Genetic divergence between genotypes was assessed using Mahalanobis D^2 statics and clusters were formed based on Tocher method as describe by Rao (1952). The cluster analysis led to the formation of seven clusters. Cluster I had the maximum number of genotypes *i.e.,* 45 followed by cluster II (15) and Cluster IV (9). Cluster III, V, VI, VII had only single genotype each (Table 2).

Intra-cluster distance was maximum in cluster II (76.15) followed by cluster IV (68.10) and cluster I (66.00). Cluster I comprised of more than half of the genotypes used in the investigation but, the intra-cluster distance was relatively less. So, it may be possible that most of the genotypes used in this study had common parentage. There was no intra-cluster distance in cluster III, V, VI and VII because they had only single genotype each. Inter-cluster distance was maximum between cluster IV and cluster VII (178.95) followed by cluster II and cluster VII (166.98). These results clearly illustrate that the only genotype (IC 436598) present in cluster VII was highly diverse. Genotype IC 436598 can be hybridized with the genotypes in cluster IV and II to produce heterotic segregants. Inter-cluster distance was found minimum between cluster III and cluster IV (56.76) as shown in Table 3. The low inter-cluster distances indicate that the genotypes of these clusters had a close genetic relationship and hence, the heterotic transgressive segregants are less likely to be obtained by hybridization. Dendrogram showing the clustering pattern of different sorghum genotypes is

presented in Figure 1. Ahalawat *et al*. (2018) got similar results when they assessed genetic divergence between 30 forage sorghum genotypes using D^2 analysis. They had recorded eleven agro-morphological characters *viz*., days to 50% flowering, plant height (cm), leaf breadth (cm), leaf length (cm), leaf area (cm^2), stem girth (mm), leaves per plant, leaf stem ratio, total soluble solids (%), protein content (%) and green fodder yield (q/ha), leading to genotype grouping into six clusters.

Cluster wise mean of all traits was calculated that is presented in Table 4. Cluster I showed high mean for traits *viz*. number of leaves/plant (24.11), green fodder yield (8.25), dry fodder yield (2.46) and low HCN content (75.61). The genotypes in cluster I can be further used in breeding programme as they were having high fodder yield with low HCN content. Moreover, indirect selection form cluster I for high fodder yield is also possible via number of leaves/plants that is positively correlated with fodder yield (Jain and Patel, 2016; Deep *et al*., 2019).

The Cluster II had low mean value for green fodder (4.07) and dry fodder yield (1.15) so, genotypes in these clusters can be considered agronomically inferior. Cluster III had high mean value for plant height (233.71), number of tiller/ plant (3.14), days to 50 per cent flowering (83.77) and TSS content (10.45). Single genotype (IC 484781) in cluster III can be used in hybridization programme for production of tall progenies. Cluster IV showed high mean value for plant height (228.00), green fodder yield (8.28) and dry fodder yield (2.40). Therefore, genotypes in cluster

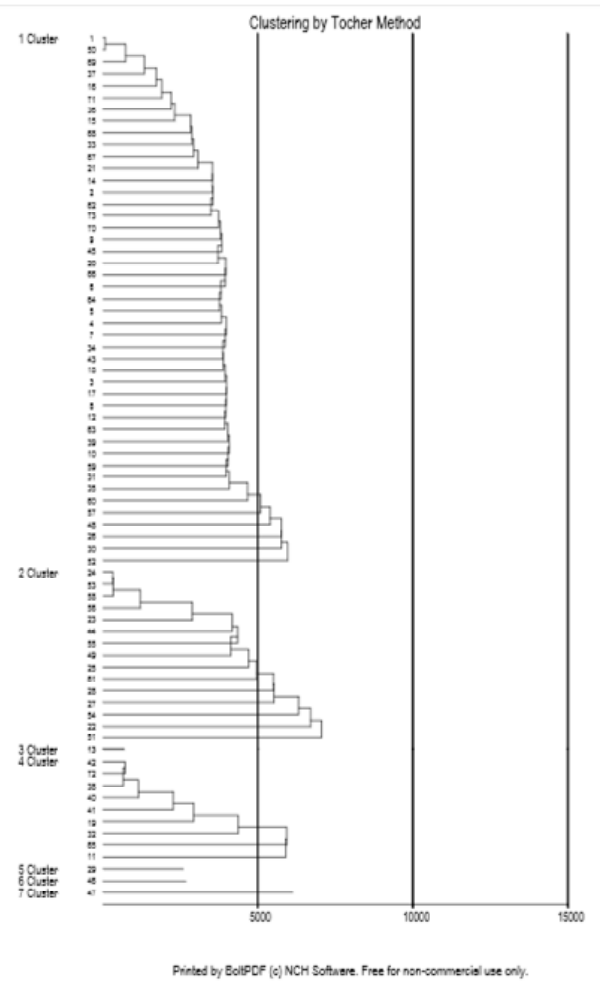

Fig. 1: Dendrogram showing the clustering pattern of different sorghum genotypes

Table 1: Treatment MMS, Mean and Range of traits under present investigation

S.No.	Characters	Range (Min.-Max.)	Mean	Treatment MSS
1	Days to 50 % flowering	67.13 – 85.91	79.41	93.42**
2	Number of tillers/plants	1.99 – 4.11	2.65	0.67**
3	Plant height (cm)	74.32 – 274.59	191.54	5584.73**
4	Leaf length (cm)	50.90 – 84.78	69.41	126.02**
5	Leaf breadth (cm)	4.56 – 8.61	6.46	2.13**
6	Number of leaves per plant	13.98 – 31.45	23.06	47.28**
7	Leaf: stem ratio	0.25 – 0.52	0.36	0.012**
8	Stem diameter (cm)	0.82 – 1.51	1.18	0.088**
9	GFY (kg/plot of 1.2 m^2)	2.06 – 15.20	7.33	29.06**
10	DFY (kg/plot of 1.2 m^2)	0.53 – 4.38	2.13	3.03**
11	Protein (%)	5.93 – 11.00	9.00	3.41**
12	HCN (mg/kg FW)	56.32 – 178.83	92.53	3237.46**
13	TSS (^{0}Brix)	5.36 – 14.21	8.90	9.37**
14	Zinc (µg/g DM)	10.91 – 18.87	15.17	10.75**
15	Copper (µg/g DM)	1.48 – 7.96	5.60	9.16**
16	Manganese (µg/g DM)	19.10 – 54.93	30.98	118.40**
17	Iron (µg/g DM)	173.75 – 382.68	247.22	4262.18**
18	IVDMD (%)	40.27 – 54.00	47.25	46.64**

GFY-Green Fodder Yield, **DFY**- Dry Fodder Yield, **TSS**- Total Soluble Solids, **HCN**- Hydrocyanic Acid, **IVDMD-** In-Vitro Dry Matter Digestibility, **=significant at 1%, *=significant at 5%

IV were agronomically superior and should be used further for forage yield improvement. Cluster V had average mean value for most of the traits. Cluster VI had high mean value for leaf length (79.97), leaf stem ratio (0.45), copper content (7.88) and IVDMD (52.16), whereas it had low mean value for green (4.02) and dry fodder yield (1.10). Cluster VII showed high mean value for leaf breadth (7.33), stem diameter (1.44), protein content (10.99), zinc content (16.76), manganese content (36.69) and iron content (382.69).Two genotypes present in cluster VI (IC

Table 2: Cluster membership profile of forage sorghum genotypes

S.No	Number of genotypes	Genotypes
Cluster 1	45	HJ 541, SSG 59-3, HC 308, HJ 513, IS 40219, IS 40264, IS 40775, IS 1098, IS 1328, IS 4523, IC 485251, IC 484601, IC 587860, IC 587867, IC 587867 -1, IC 587874, IC 587892, IC 485074, IC 484982, IC 484883, IC 484572, IC 485098, IC 485145 -1, IC 485145, IC 484918, IC 484347, IC 484490, IC 585159, IC 395771, IC 413292, IC 527022, IC 397227, IC 436839, IC 144858, IC 144869, IC 144888, IC 285835, IC 285858, IC 285875, IC 255879, IC 285895, IC 285896, IC 285918, IS 651, SSG 234-2, IS 720, PSC 2-1
Cluster 2	15	IC 240878, IC 144842, IC 144876, IC 144861, IC 240847, IC 395776, IC 144858, IC 249108, IC 484975, IC 285848, IC 484984, IC 485017, IC 144850, IC 240844, IC 397242
Cluster 3	1	IC 484781
Cluster 4	9	IC 585238, PSC 2, IC 585136, IC 585183, IC 585186, IC 587881, IC 485098, IC 285892, IS 2984
Cluster 5	1	IC 485039
Cluster 6	1	IC 436522
Cluster 7	1	IC 436598

Table 3: Inter and intra – cluster distances in sorghum genotypes

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Cluster 7
Cluster 1	**66.00**						
Cluster 2	108.32	**76.15**					
Cluster 3	82.37	123.97	—-				
Cluster 4	97.5	130.82	56.76	**68.10**			
Cluster 5	129.42	102.95	127.76	152.36	—-		
Cluster 6	123.97	94.89	93.31	109.94	80.25	—-	
Cluster 7	165.25	166.98	145.43	178.95	78.33	125.78	—-

Table 4: Cluster means for different characters in sorghum genotype

Characters	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Cluster 7
Days to 50 % flowering	79.34	81.52	**83.77**	75.20	76.30	83.74	83.51
Number of tillers/plants	2.63	2.92	**3.14**	2.28	3.00	2.37	2.47
Plant height (cm)	206.67	128.15	**233.71**	228.00	137.41	149.93	186.97
Leaf length (cm)	70.24	64.50	74.55	71.81	64.69	79.97	73.39
Leaf breadth (cm)	6.51	6.33	5.33	6.46	6.32	7.02	7.53
Number of leaves/plants	**24.11**	20.89	23.96	22.56	20.51	17.96	19.75
Leaf: stem ratio	0.36	0.44	0.32	0.31	0.42	0.45	0.34
Stem diameter (cm)	1.18	1.14	1.21	1.18	1.20	1.42	1.44
GFY (kg/plot of 1.2 m^2)	**8.25**	4.07	6.49	8.28	4.33	4.02	8.08
DFY (kg/plot of 1.2 m^2)	**2.46**	1.15	1.69	2.40	1.23	1.10	2.47
Protein (%)	8.78	8.96	10.66	9.66	7.98	7.54	**10.99**
HCN (mg/kg FW)	**75.61**	101.20	137.47	137.24	131.86	173.15	156.93
TSS (^{0}Brix)	9.05	8.52	**10.45**	8.64	7.83	7.32	8.00
Zinc (µg/g DM)	15.01	15.59	15.41	15.11	14.64	15.93	**16.76**
Copper (µg/g DM)	5.45	5.86	6.69	5.36	5.88	**7.88**	7.18
Manganese (µg/g DM)	30.55	32.80	24.50	31.15	30.37	25.74	**36.29**
Iron (µg/g DM)	248.32	246.92	247.27	216.09	329.32	264.88	**382.69**
IVDMD (%)	47.12	48.49	50.12	46.12	42.18	**52.16**	41.77

GFY-Green Fodder Yield, **DFY**- Dry Fodder Yield, **TSS-** Total Soluble Solids, **HCN**- Hydrocyanic Acid, **IVDMD-** In-Vitro Dry Matter Digestibility

Table 5: Grouping of genotypes based on midrib colour

Midrib colour	Number of genotypes	Genotypes
Green/Dull	30	HJ 541, SSG 59, HC 308, IS 40264, IS 4523, IS 2984, IC 485251, IC 587881, IC 240844, IC 240847, IC 240878, IC 484982, IC 484984, IC 484490, IC 585159, IC 395776, IC 436522, IC 527022, IC 397242, IC 436839, IC 144850, IC 144858, IC 144861, IC 144869, IC 144876, IC 285835, IC 255879, IS 720, PSC 2, PSC 2-1
White	43	HJ 513, IS 40219, IS 40775, IS 1098, IS 1328, IC 484781, IC 484601, IC 587860, IC 587867, IC 587867-1, IC 587874, IC 587892, IC 485074, IC 484975, IC 485017, IC 485039, IC 484883, IC 484572, IC 485098, IC 485145 -1, IC 485145, IC 484918, IC 484347, IC 585136, IC 585183, IC 585186, IC 585238, IC 395771, IC 413292, IC 436598, IC 249108, IC 397227, IC 144842, IC 144888, IC 285848, IC 285858, IC 285875, IC 285892, IC 285895, IC 285896, IC 285918, IS 651, SSG 234-2

436522) and cluster VII (IC 436522) were superior with respect to most of the quality traits, therefore they should be hybridized with high yielding genotypes to develop verities having higher yield as well as quality. Such confirmatory results were also obtained by Meena *et al.,* 2016, Doijad *et al.,* 2016 and Chikuta *et al.,* 2015 in forage sorghum.

In the present investigation, besides various components of fodder yield and quality, genotypes were also classified according to midrib color as shown Table 5. Green/Dull midrib color is an indicator of juiciness of stalk and better palatability of green fodder whereas, white ones are known to be non-sweet with pithy stalk and have poor palatability (Rangaswami *et al*., 1937; Teshome *et al*., 1997).

From the ongoing discussion it can be depicted that most of the genotypes used in present investigation have common ancestry as more than half of them grouped into single cluster. Moreover, genotypes with high value for quality traits clustered separately from those with high fodder yield.The high fodder yielding genotypes *viz*., HJ 541, HJ 513, SSG 59-3, IC 485017, IS 720, IS 392442, IC 240847, IC 249108 were grouped together in cluster I and II whereas, genotypes showing high value for quality parameters *viz*., IC 436522 and IC 436598 were present in cluster VI and VII respectively. So, the genotypes in these clusters can be used for selection and/or hybridization purpose to further improve the fodder yield and quality in forage sorghum.

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