International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2017; 5(4): 337-341

Received: 05-05-2017
Accepted: 06-06-2017

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D^2 analysis in forage Sorghum [*Sorghum bicolor* (L.) Moench]

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Abstract
A field experiment comprised of 60 genotypes of forage sorghum [*Sorghum bicolor* (L.) Moench] was laid out-in Randomized Block Design replicated thrice, at the experimental farm of Main Forage Research Station, Anand Agricultural University, Anand during *kharif*-2016-17 to investigate D^2 analysis in forage sorghum. Mahalanobis D^2 statistics revealed considerable genetic diversity among the 60 genotypes of forage sorghum. The composition of cluster was done on the basis of relative magnitude of D^2 values following Tocher's method (Rao, 1952) [6]. The genotypes grouped into 5 clusters. Cluster I contains forty genotypes followed by sixteen in cluster II, while cluster III and V were contain solitary genotype and cluster IV had only two genotypes. D^2 analysis will be helpful in designing breeding programmes to obtain high yielding genotypes in sorghum for forage yield.

Keywords: D^2 analysis, Clusters

1. Introduction
Livestock is most important component of rural economy as well as back bone of Indian agriculture characterized by mix farming system involving crop and animal enterprises. Like green revolution, India is contemplating for white revolution, which is possible only with adequate supply of nutritious feeds and sufficient fodders. In dairy production, the cost of feed constitutes about 60-65% of the total cost of milk production. To reduce the cost of milk production, continuous supply of green fodder to the animal is necessary. There is need for continuous and steady supply of green fodder to increase milk production of animal under different intensive programmes executed for the success of white revolution, in the state. The livestock population in India is 512.05 million and expected to grow at the rate of 1.23% in the coming years (Anonymous, 2012) [2]. Out of total cultivated area, only 4 % area is covered by fodder cultivation. In recent years, India is facing an acute shortage of feeds and fodder. Our green forage and dry forage availability is 405.9 million tonnes and 473 million tonnes respectively against the requirement of 1034 million tonnes and 630 million tonnes, which shows 64.21 % and 24.81 % deficit, respectively (Anonymous, 2011-12) [1]. Therefore, it is urgent need to emphasize on increasing in forage crops production per unit area through evolving high yielding and improved variety of forage crops as well as innovative forage production technology. Therefore, target could be achieved by developing the varieties or hybrids of forage crop giving high yield per unit area and better quality.
Sorghum [*Sorghum biocolor* (L.) Moench] is commonly known as "Great millet" due to larger size of grain among millets and vast area under it. It is the fifth most important crop in the world. It is a member of *Poaceae* (*Graminaeae*) family with chromosome number 2n=2x=20. It belongs to the genus *sorghum*. Sorghum is a native plant of Africa and South Asia (House, 1995). Its centre of origin is Ethiopia (Africa). Assessment of genetic diversity in germplasm can facilitate classification and identification of diverse heterotic group with possible breeding value in manifestation of breeding potential of genotype in specific breeding programme. Mahalanobis (1936) [4] generalized distance analysis is useful in measuring genetic diversity between two population. In view of this, an attempt was made to assess the genetic divergence present for forage yield and its components traits in different sorghum accessions so as to identify superior genotypes of sorghum which can be utilized for future breeding programmes.
Precise information on the nature and degree of genetic diversity help plant breeder in selecting the parents for targeted hybridization. It provides the raw materials from which desirable alleles for improved agronomic traits of interest can be selected and subsequently incorporated into elite lines.

Materials and methods

The proposed investigation was carried out at Main Forage Research Station, A.A.U., Anand, during *kharif* season of the year 2016 with the objective to obtain information on the nature and degree of genetic diversity from 60 genotypes of forage sorghum. Anand is situated at $22^0$35' North Latitude and $72^0$55' East longitude and 45.11 m above the mean sea level. The soil of experimental field is sandy loam, which is locally known as "*Goradu"* Soil. It is alluvial in origin, deep, well drained and has fairly good moisture holding capacity. It is poor in organic matter, medium in available phosphorous and rich in available potash. It responds well to irrigation and nitrogen application. The experiments were raised in randomized block design with three replications during *kharif* 2016-17. Each entry was accommodated in single row spaced at 30 cm apart with plant-to-plant spacing of 10 cm. All the recommended agronomic practices and plant protection measures were followed to raise the healthy crop. Observations were recorded from five randomly selected plants for each genotypes in all the replications for sixteen characters *viz.*, Days to 50 % flowering, plant height at 50% flowering (cm), number of tillers/plant at harvest, number of leaves/plant at harvest, leaf length (cm) at harvest, leaf width (cm) at harvest, leaf: stem ratio at harvest, stem thickness (cm) at harvest, green forage yield (g)/ plant, dry matter content (%), dry matter yield (g)/plant, crude protein content (%), crude protein yield (g)/plant, HCN content (ppm), neutral detergent fiber content (NDF %) and crude fiber content (CF %). Morphological diversity analysis of all the sixteen characters under study was made on the basis of mean values. The statistical analysis was carried out by using the computer facility available at the Department of Agricultural Statistics, B. A. College of Agriculture, Anand Agricultural University, Anand. The data recorded for various characters were subjected to analysis of variance and Mahalanobis D^2 statistics for estimation of morphological diversity with the help of IndoStat software.

Grouping of the genotypes in different clusters was done by using Tocher's method (Rao, 1952) [6]. The criterion used in clustering in this method is that any two genotypes belonging to the same cluster should on an average show a smaller D^2 values than those belonging to different clusters. To start with, two genotypes having the lowest D^2 values between them were considered, to which a third genotype having next smallest D^2 value from these genotypes was added. Next, the nearest fourth genotype was considered and so on. At a certain stage, when it was felt that the inclusion of a genotype results in an abrupt increase in the average D^2 value of the genotypes (for that cluster), that genotype was not included in the cluster. In a similar way the other clusters were formed. This procedure was continued till all the genotypes were included in one cluster or the other.

Results and discussion

The success of selection depends on the presence of wide genetic diversity in experimental material. The knowledge regarding the extent of variability and genetic diversity is of much importance while making improvement in a complex trait like yield. Therefore, while improving forage yield, selection of parents having wide divergence for number of characters is of prime importance, which can be assessed by D^2-statistics developed by Mahalanobis (1936) [4]. Several plant breeders used the D^2 technique for selection of divergent parents and their further exploitation in hybridization programmes which helped breeders in genetic interpretation of material under investigation. In view of this, attempt was made to assess the genetic divergence present for forage yield and its component traits in different forage sorghum accessions so as to identify superior genotypes of forage sorghum which can be utilized for future breeding programme.

The analysis of variance for individual characters revealed significant difference among genotypes. Wilk's criterian showed significant difference between genotypes for pooled effect of 16 traits. (Wilk's criteria X^2=4104.4110 at 944 degree of freedom). Hence, further analysis was done to calculate D^2 value range from11.42 (between PB- 75 and PB-253 genotypes) to 5338.87 (between IS-31693 and SS-96-787 genotypes). This indicated the presence of high genetic diversity among the genotypes for all the characters. Based on observation of 16 characters, the Mahalanobis D^2 statistic was computed for all possible pairs of 60 genotypes under study. The theoretical concept behind cluster formation is that the genotypes grouped into the same cluster presuming are less diverse from each other than those belonging to different clusters which have large distance. The genotypes of different clusters can be utilized for better exploitation of heterosis in hybridization. Therefore, selection of parents from diverse clusters is perquisite for fruitful effects.

Distribution of Genotypes into Cluster

The composition of cluster was done on the basis of relative magnitude of D^2 values following Tocher's method (Rao, 1952) [6] on the assumption that the genotype within the cluster had D^2 values among themselves than those from group's belonging to different clusters. The 60 genotypes of forage sorghum were grouped into five clusters and are presented in table 1. Cluster I was the largest having 40 genotypes. The second largest cluster II had 16 genotypes, while cluster IV have two genotypes, whereas cluster III and V were mono genotypic. The clustering pattern revealed that in general, genotypes from same origin showed no tendency to be in the same cluster. Looking to the pattern of genotype distribution into different clusters in the present study, it appeared that geographical distance had no relation with the genetic divergence as the genotypes from same source had fallen into different clusters.

Table 1: Distribution of 60 genotypes of forage sorghum in different clusters on the basis of D^2 statistics.

Sr. no.	Cluster	Number of Genotype	Name of the Genotype
1	I	40	Piper-7, Piper-66, Piper-96, PB-4, PB-19, PB-26, PB-34, PB-39, PB-53, PB-56, PB-64, PB-75, PB-78, PB-84, PB-92, PB-100, PB- 106, PB-116, PB-119, PB-126, PB-131, PB-173, PB-184, PB-186, PB-212, PB-253, PB-257, IS-3192, IS-3201-1, IS-3284, IS-3332, IS-3342, IS-8347, DSIS-4889, IS-21465, SS-96-787, SSG-59-3, CoFS-29, CoFS-31, GAFS-11.
2	II	16	Piper-14, Piper-33, Piper-52, Piper-95, PB-76, PB-226, PB-266, PB-271-2, IS-3373, IS-9840, IS-K 5389, SRF-323, PC-23, S-1049, C-10-2, GAFS-12.
3	III	01	PB-113
4	IV	02	PB-147, IS-31693
5	V	01	PB-146

Table 2: Average intra-cluster (diagonal) and inter-cluster (off diagonal) value for different genotypes of forage sorghum.

Clusters	I	II	III	IV	V
I	228.70	781.36	414.23	2822.38	2424.91
II		206.68	638.65	978.23	923.43
III			0.0	2154.56	1992.43
IV				368.28	639.76
V					0.0

Intra and inter cluster distance

The average intra (diagonal) and inter cluster (off-diagonal) D^2 values are presented in Table 2. A study of data revealed that the inter cluster distance- D^2 value ranged from 414.23 to 2822.38. The maximum inter cluster distance was observed between cluster I and IV (2822.38) followed by cluster I and V (2424.91), cluster III and IV (2154.56) and cluster III and V (1992.43). The minimum inter cluster distance (D^2 =414.23) was observed between the cluster I and IV, followed by cluster I and III (638.65), cluster IV and V (639.76), cluster I and II (781.36), cluster II and V (923.43), cluster II and IV (978.23). Intra cluster distance (D^2) ranges from 0.0 to 368.28 at intra cluster level. Cluster IV had the highest value (D^2 = 368.28) which was followed by cluster I (D^2 = 228.70). The intra cluster distance was zero for cluster III and IV as these two clusters composed of single genotype.

In the present investigation, the inter cluster distance was higher than intra cluster distance which indicated substantial diversity among the genotypes and there may be a greater opportunity for obtaining the rare but superior segregants from crosses between more divergent genotypes. Similar results were also obtained by earlier investigators (Swami *et al.,* 2015; Jain and Patel, 2013; and Mohanraj *et al.,* 2006) [7, 3, 5].

Cluster Mean for Different Characters

The cluster mean for 16 characters are presented in table 3. A considerable inter cluster variation was observed among the cluster mean for most of the characters. Green forage yield per plant was the highest in cluster III (122 g), followed by cluster I (79.07 g) and cluster V (77.33 g) with average cluster mean of 78.04 g. Cluster I exhibited highest mean value for crude fibre (24.60 %) with the lowest mean value for HCN content (30.33 ppm) and stem thickness (0.72 cm). While, cluster II showed moderate mean value for all the characters and lowest cluster mean value for plant height at 50% flowering (177.84 cm) and crude protein yield per plant (1.37 %). Cluster III depicted the highest cluster mean value for all the traits, except stem thickness and HCN content. While cluster IV revealed highest cluster mean value for the crude protein content (8.60 g) and HCN content (120.29 ppm) and moderate to low cluster mean value for remaining traits. Cluster V showed the highest cluster mean value for leaf: stem ratio (2.75), stem thickness (1.85 cm) and NDF content (68.89 %) However, the lowest cluster mean value for the leaf length, leaf width, dry matter content and dry matter yield per plant showed moderate mean value for remaining traits. The mean values for other important yield attributing traits *viz.,* crude protein content (8.60%) and HCN content (120.29 ppm) were depicted appreciably higher for cluster IV and crude fibre content (24.60 %) was significantly higher for cluster I and cluster V (23.90 %). Thus, the considerable degree of divergence existed in the present forage sorghum genotypes. The maximum amount of heterosis is expected from the crosses with accessions belonging to the most divergent clusters as have also been reported earlier. (Jain and Patel, 2013) [3].

The analysis of variance for each character was carried out using mean of the 60 genotypes. Estimates of inter and intra cluster variances, along with ratio (R^2) of inter cluster variances to the total variance and inter cluster coefficient of variation (CV_b) for the 16 characters (Table 3) were worked out. Maximum value of R^2 (0.96) was observed for HCN content followed by number of leaves per plant (0.69), leaf width (0.69), dry matter yield per plant (0.69) and crude protein yield per plant (0.68) which indicates these traits had higher contribution for genetic diversity among the all traits. The days to 50 % flowering (0.50), green forage yield per plant (0.49), dry matter content (0.43), crude protein content (0.39), plant height at 50% flowering (0.34) and crude fiber content (0.21) had moderate R^2 values. The lowest R^2 values were observed for leaf length (0.063) followed by number of tillers per plant (0.10) and NDF content (0.13). It indicated that these traits had low contribution in genetic diversity. The leaf: stem ratio and stem thickness did not contribute for the genetic divergence because the value of R^2 value was not estimated due to negative variance.

Inter-cluster coefficient of variance was maximum for HCN content (145.41), followed by dry matter yield per plant (42.14), crude protein yield per plant (40.37), green forage yield per plant (20.69), number of leaves per plant (20.12), leaf width (17.37), dry matter content (15.80) and days to 50 % flowering (12.26).The minimum CV_b value was observed for NDF content (1.88) followed by leaf length (2.65). These traits manifested higher CV_b values indicating an important role in the genetic discrimsination of the genotypes included under study.

Intercrossing of divergent groups would lead to wide genetic base in the base population and greater opportunities for crossing over to occur, which inturn may release hidden variability by breaking close linkage. The progenies derived from such crosses are expected to show wide variability, providing greater scope for isolating transgressive segregants in the advanced generations. Hence, these genotypes may be used repeatedly in the crossing programmes to recover transgressive segregants, which can be either released as a variety or can be utilized in the genetic enhancement of forage sorghum crop.

Table 3: Cluster means of different characters in forage sorghum

Cluster	Days to 50 % flowering	Plant height (cm)	Number of tillers per plant	Number of leaves per plant	Leaf length (cm)	Leaf width (cm)	Leaf : stem ratio	Stem thickness (cm)	Green forage yield (g)	Dry matter content (%)	Dry matter yield (g) per plant	Crude protein content (%)	Crude protein yield (g) per plant	HCN Content (ppm)	NDF (%)	CF (%)
I	61.60	182.50	1.88	11.34	66.70	3.82	1.77	0.72	79.07	26.84	21.24	7.84	1.66	30.33	66.55	24.60
II	56.02	177.84	1.81	10.87	67.02	3.45	1.76	0.73	73.43	24.37	17.72	7.70	1.37	70.47	67.49	23.89
III	71.00	210.00	2.05	17.53	62.13	4.79	1.57	0.81	122.00	34.46	42.09	7.56	3.18	51.15	63.37	22.87
IV	60.00	178.90	1.32	10.54	68.73	4.23	1.58	0.90	72.66	28.07	21.00	8.60	1.79	120.29	66.53	24.18
V	56.00	208.00	1.81	11.11	59.40	3.18	2.75	1.85	77.33	21.90	16.96	8.28	1.40	107.52	68.89	23.90
S. Em. ±	3.05	7.47	0.22	0.63	2.78	0.18	0.10	4.01	6.79	1.96	2.36	0.21	0.18	4.90	1.34	1.03
CD at 5 %	8.46	20.73	NS	1.74	NS	0.50	0.28	0.11	18.83	5.45	6.55	0.59	0.52	13.60	NS	NS
CV %	12.23	9.89	29.65	13.45	10.06	11.64	13.98	12.87	20.96	18.02	27.68	6.54	28.34	25.87	4.84	10.19
R^2	0.50	0.34	0.10	0.69	0.063	0.69	-	-	0.49	0.43	0.69	0.39	0.68	0.96	0.13	0.21
CV_b %	12.26	7.21	10.13	20.12	2.65	17.37	-	-	20.69	15.80	42.14	7.91	40.37	145.41	1.88	5.37

R^2: Ratio of the inter-cluster variance to the total variance, NS: Non-Significant, - : Not estimated due to –ve variance, CV_b : Inter-cluster coefficient of variation

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