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Research Article

Genetic diversity analysis of geographically diverse landraces and wild accessions in sorghum

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Abstract
Landraces are highly dynamic populations with historical origins. Several factors have influenced the evolution of landraces and some of them were selection by farmers, natural selection, gene-flow from various neighboring populations, genetic drifts, *etc*. In this study, we phenotyped 36 landraces and wild populations of sorghum conserved at ICRISAT genebank, Hyderabad, India, to assess the diversity among the populations of sorghum. Since landraces are diverse populations, we phenotyped more plants (over 100 plants) in each accession for nine economically important quantitative traits, thus capturing the entire variability. Our study revealed that the clustering pattern based on D^2 statistics grouped the 36 accessions into 5 clusters, in which cluster 1 holds the maximum number of 15 accessions and cluster 3 with the least (2 accessions). Minimal intra cluster distance (3.66) was observed in cluster 3 followed by cluster 5 (9.52), while the maximum intra cluster distance was found in cluster 2 (12.50). The highest inter cluster distance was found between clusters 1 and 4 (16.29) indicating the presence of wide diversity between accessions of these two clusters. Accessions in cluster 1 were early maturing (67 days after sowing, DAS) while those in cluster 4 were late maturing (129 DAS) and high yielding (36.9 g). Results from this study provide information about the diversity of landraces and wild sorghum populations for their utilization in crop improvement

Key words
Sorghum, Clustering, D^2 Mahalanobis, Diversity, Landraces

INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is a staple food crop for millions of the poorest and most food-insecure people in the semi-arid tropics. It is a multipurpose crop owing to its usage as food, feed, fuel, and bioenergy (Blümmel *et al.* 2003; Torrecillas, Cantamutto, and Bertoia 2011; Chen et al. 2014). Sorghum ranks fifth among cereals in terms of area (42.14 M ha) and production (59.34 M t) in 2018 (FAOSTAT 2020), and it is grown in over 100 countries in the arid and semi-arid regions of the world. The improved hybrids and varieties of sorghum were reported to be less diverse compared to its wild weedy relatives and landraces (Jordan *et al*. 2003, 1998; Mace *et al.* 2013; Murray *et al*. 2009; Mutegi *et al.* 2011; Smith *et al*. 2010). This low diversity of crop cultivars is mainly because of the low use of existing variability in crop improvement. Higher uniformity may result in an increased risk of crop vulnerability, such as crop failure due to insect pests and disease epidemics or unpredictable climatic effects (Upadhyaya and Vetriventhan, 2018). Globally, a significant number of sorghum germplasm accessions (over 230000 accessions) have been conserved in genebanks, that can be potentially utilized to enhance stress tolerance, quality, and productivity of sorghum (Upadhyaya and Vetriventhan, 2018).

Knowledge about germplasm diversity can support breeders in selecting desirable and diverse accessions for their utilization in crop improvement programmes. Each landrace accession should be considered as a continually evolving population, progressing through various forces of natural and farmers' selection. Data from a small number of randomly selected plants in landrace accessions may

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not be a good representation of a structured population. Thus, we phenotypically characterized 36 geographically diverse accessions of sorghum (including landraces and wild accessions), by recording data from the maximum number of plants possible for assessing the diversity among accessions.

MATERIALS AND METHODS

A total of 36 geographically diverse accessions (**Table 1**) which included 31 landraces and 5 wild accessions of sorghum conserved at ICRISAT genebank's collection, were used for this present study. IS 33844 was used as control and it is the most popular sorghum variety widely grown during *rabi* (postrainy) season in India. The field experiment was conducted in the postrainy season of 2018 at ICRISAT, Patancheru, India. Accessions were sown in black soil and each accession occupied three rows of 9 m length with a plant-to-plant spacing of approximately 10 cm and 75 cm between rows. Data on nine quantitative traits namely, days to flowering, plant height (cm), leaf blade length and width (cm) (recorded on the third leaf from top), panicle exertion (cm), panicle length (cm), panicle width (cm), panicle(s) weight per plant (g) and grain yield per plant (g) were recorded following sorghum crop descriptors (IBPGR and ICRISAT, 1993). Thus, to capture the entire variability in each accession, data were collected from a large number of randomly selected plants in each accession, and within each accession, the plant count varied from 115 to 234. Mean, standard deviation, variance, and range were estimated, and mean data was used for D^2 statistic (Rao 1952) based genetic diversity assessment. For grouping into clusters, the minimum generalized distance based on Tocher's method (Rao 1952) was utilized.

Table 1. List of sorghum accessions used in this study and their passport information

Accession Number	Race	Sub-species	Biological status	Country of origin	Region
IS 2134	Guinea-kafir	*bicolor*	Traditional cultivar/Landrace	Ethiopia	Africa
IS 2153	Bicolor	*bicolor*	Traditional cultivar/Landrace	Nigeria	Africa
IS 3399	Kafir	*bicolor*	Traditional cultivar/Landrace	Botswana	Africa
IS 11005	Wild	*drummondii*	Wild	Ethiopia	Africa
IS 14010	Caudatum-bicolor	*bicolor*	Traditional cultivar/Landrace	South Africa	Africa
IS 14485	Wild	*verticilliflorum*	Wild	Sudan	Africa
IS 18833	Wild	*verticilliflorum*	Wild	Malawi	Africa
IS 22407	Guinea-durra	*bicolor*	Traditional cultivar/Landrace	Sudan	Africa
IS 22428	Wild	*drummondii*	Wild	Sudan	Africa
IS 25476	Caudatum	*bicolor*	Traditional cultivar/Landrace	Burundi	Africa
IS 27325	Guinea-bicolor	*bicolor*	Traditional cultivar/Landrace	Burkina Faso	Africa
IS 29508	Kafir	*bicolor*	Traditional cultivar/Landrace	Lesotho	Africa
IS 29605	Kafir-bicolor	*bicolor*	Traditional cultivar/Landrace	Lesotho	Africa
IS 31637	Durra-caudatum	*bicolor*	Traditional cultivar/Landrace	Rwanda	Africa
IS 34283	Guinea	*bicolor*	Traditional cultivar/Landrace	Zimbabwe	Africa
IS 35474	Guinea-kafir	*bicolor*	Traditional cultivar/Landrace	Namibia	Africa
IS 40031	Guinea-caudatum	*bicolor*	Traditional cultivar/Landrace	Uganda	Africa
IS 40161	Durra	*bicolor*	Traditional cultivar/Landrace	Mali	Africa
IS 12919	Durra-bicolor	*bicolor*	Traditional cultivar/Landrace	Guadeloupe	America
IS 12965	Caudatum	*bicolor*	Traditional cultivar/Landrace	Cuba	America
IS 13215	Durra	*bicolor*	Traditional cultivar/Landrace	Guatemala	America
IS 1128	Bicolor	*bicolor*	Traditional cultivar/Landrace	India	Asia
IS 2348	Kafir-caudatum	*bicolor*	Traditional cultivar/Landrace	Indonesia	Asia
IS 8330	Caudatum	*bicolor*	Traditional cultivar/Landrace	India	Asia
IS 10897	Wild	*drummondii*	Wild	India	Asia
IS 18234	Guinea-caudatum	*bicolor*	Traditional cultivar/Landrace	Bangladesh	Asia
IS 22606	Guinea	*bicolor*	Traditional cultivar/Landrace	Sri Lanka	Asia
IS 32252	Durra-caudatum	*bicolor*	Traditional cultivar/Landrace	Yemen	Asia
IS 32263	Durra	*bicolor*	Traditional cultivar/Landrace	Yemen	Asia
IS 35217	Caudatum-bicolor	*bicolor*	Traditional cultivar/Landrace	Pakistan	Asia
IS 40238	Durra-bicolor	*bicolor*	Traditional cultivar/Landrace	India	Asia
IS 13211	Bicolor	*bicolor*	Traditional cultivar/Landrace	Spain	Europe
IS 21858	Guinea-caudatum	*bicolor*	Traditional cultivar/Landrace	Russia	Europe
IS 13065	Durra-bicolor	*bicolor*	Traditional cultivar/Landrace	Australia	Oceania-Pacific
IS 13068	Kafir-durra	*bicolor*	Traditional cultivar/Landrace	Australia	Oceania-Pacific
IS 33844	Durra	*bicolor*	Traditional cultivar/Landrace	India	Asia

RESULTS AND DISCUSSION

The mean, standard deviation, variance, and range were estimated over the 36 accessions and presented in **Table 2**. The range estimates showed that the selected accessions were diverse for all the phenotypic traits studied, including for days to flowering which varied from 53 to 92 days after sowing (DAS) with a mean of 71 DAS. Plant height varied from 98 to 311 cm with a mean of 224 cm and grain yield varied from 2 to 48 g per plant. The higher standard deviation of within and among accessions indicating a considerable variability within and among accessions.

The hierarchal clustering based on Mahalanobis's D^2 distances using Tocher's method grouped the 36 accessions into five clusters indicating the presence of considerable diversity in the studied accessions. A maximum number of accessions were found in cluster 1 (15 accessions) followed by cluster 2 and 5, each containing 8 and 7 accessions, respectively (**Table 3**), while cluster 3 and 4 had 2 and 4 accessions, respectively. Ethiopian regions of Africa, being the primary origin of sorghum, high diversity was observed for the African accessions, which resulted in the distribution of African accessions to all the five clusters. Also, the clustering of African accessions with Asian accessions shows their similarity, diffusion, movement, and historical domestication of sorghum landraces from Africa towards Asia and the Indian subcontinent. However, the clustering pattern showed no grouping of accessions into clusters in correspondence to geographical locations, from where the accessions are collected, as reported in previous studies in sorghum (Yadav, Pahuja, and Grewal 2003; Kadam et al. 2001; Ganesamurthy, Punitha, and Elangovan 2010).

Table 2. Mean, standard deviation, variance, and range estimates of within and among accessions for nine quantitative traits

Trait	Overall			Within accessions		
	Mean	Standard deviation	Variance	Range of mean	Range of standard deviation	Range of variance
Days to flowering	71	9.85	97.10	53 – 92	2.26 – 5.57	5.11 – 31.02
Plant height (cm)	224	59.68	3561.22	98 – 311	8.93 – 47.45	79.74 – 2251.50
Leaf blade length (cm)	60	6.46	41.72	40 – 79	4.61 – 11.44	21.25 – 130.87
Leaf blade width (cm)	5.6	0.96	0.93	3.9 – 7.9	0.52 – 6.23	0.27 - 38.81
Panicle exertion (cm)	13	5	26.99	3 – 22	4.13 – 11.07	17.06 – 122.54
Panicle length (cm)	23	6.63	44.99	12 – 46	1.8 – 6.8	3.24 – 46.24
Panicle width (cm)	10	4.69	22.02	5 – 26	1.21 – 9.23	1.46 – 85.19
Panicle(s) weight per plant (g)	36	14.95	223.58	7 – 66	7.28 – 41.31	53 – 1706.52
Grain yield per plant (g)	24	12.58	158.13	2 – 48	5.8 – 33.37	33.64 – 1113.56

Table 3. D^2 clustering pattern of 36 accessions of sorghum, based on nine quantitative traits

Cluster	No of accessions clustered	Asia	Africa	America	Oceania-Pacific	Europe
Cluster 1	15	IS 1128, IS 2348, IS 8330, IS 10897 (wild)	IS 2134, IS 2153, IS 3399, IS 11005 (wild), IS 14010, and IS 29605	IS 12919 and IS 12965	IS 13065 and IS 13068	IS 13211
Cluster 2	8	IS 18234	IS 14485 (wild), IS 18833 (wild), IS 22407, IS 22428 (wild) and IS 35474	IS 13215		IS 21858
Cluster 3	2	IS 35217	IS 25476			
Cluster 4	4	IS 22606 and IS 40238	IS 27325 and IS 40031			
Cluster 5	7	IS 32252, IS 32263 and IS 33844	IS 29508, IS 31637, IS 34283 and IS 40161			

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Table 4. Average intra and inter-cluster distance (D^2) value of sorghum accessions for nine quantitative traits

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
Cluster 1	11.04	12.92	11.67	16.29	10.95
Cluster 2		12.50	9.79	12.90	11.63
Cluster 3			3.66	10.44	8.02
Cluster 4				11.55	14.11
Cluster 5					9.52

The intra and inter cluster distances in D^2 analysis among the 36 accessions (**Table 4**) indicated that cluster 3 showed the lowest intra cluster value (3.66) with only 2 accessions. Even though these two accessions were from different geographic locations (Asia and Africa), grouping of these accessions into a single cluster depicts that the accessions within this cluster were less divergent for the studied quantitative traits. Cluster 2 showed the highest intra cluster distance (12.50) followed by cluster 4 (11.55). Maximum inter cluster distance (16.29) was observed between clusters 1 and 4, indicating the accessions belonging to these groups were highly diverse and also shows their diversity to the studied traits. Following that, the highest inter cluster distance was found between clusters 5 and 4 (14.10) and between clusters 1 and 2 (12.92) showing their considerable diversity between the accessions in these clusters.

The wild accessions were found to be clustered in clusters 1 and 2. *Sorghum bicolor* has been subdivided into three subspecies: (i) subsp. *bicolor,* it includes all the domesticated grain sorghum, (ii) subsp. *drummondii*, which are derivatives of hybridization among grain sorghum and their closest wild relatives and (iii) subsp. *verticilliflorum* (earlier subsp. *arundinaceum*), which are the wild progenitors of grain sorghum. All these three subspecies belong to the primary genepool of sorghum. Two accessions of *S. bicolor* subsp. *drummondii* (IS 10897 and IS 11005) clustered in cluster 1, while in the cluster 2, one accession of *S. bicolor* subsp. *drummondii* (IS 22428) and two accessions of *S. bicolor* subsp. *verticilliflorum* (IS 18833 and IS 14485) were clustered together. This shows the close association of *S. bicolor* subsp. *drummondii* to the accessions of cluster 1. Also the grouping of both *S.* subsp. *drummondii* and *S.* subsp. *verticilliflorum* in cluster 2 shows that these accessions have phenotypic similarity for the studied traits.

The per cent contribution of each of the nine traits towards diversity (**Table 5**) showed the higher contribution of grain yield per plant (21.43%), followed by days to flowering (17.30%) and panicle(s) weight per plant (15.71%). The cluster mean values estimated over 36 accessions for nine traits showed a wide range of variation. Accessions in cluster 1 were early maturing (67 DAS) while those in cluster 4 were late maturing (129 DAS) and higher grain yielding (36.9 g) (**Table 6**).

Table 5. Percent contribution of nine quantitative traits towards diversity in sorghum

S. No.	Character	Contribution in percent
1	Days to flowering	17.30
2	Plant height (cm)	9.37
3	Leaf blade length (cm)	1.90
4	Leaf blade width (cm)	1.43
5	Panicle exertion (cm)	7.30
6	Panicle length (cm)	10.79
7	Panicle width (cm)	14.76
8	Panicle(s) weight per plant (g)	15.71
9	Grain yield per plant (g)	21.43

Table 6. Cluster means for nine quantitative characters studied in sorghum

Cluster	Days to flowering	Plant height (cm)	Leaf blade length (cm)	Leaf blade width (cm)	Panicle exertion (cm)	Panicle length (cm)	Panicle width (cm)	Panicle(s) weight per plant (g)	Grain yield per plant (g)
1	67	186.73	59.87	5.32	13.26	24.00	9.45	27.83	16.63
2	74	242.08	58.55	5.05	17.20	24.73	12.13	38.42	26.80
3	72	276.25	57.05	6.08	9.72	18.50	9.21	40.69	29.65
4	83	287.40	67.75	6.16	13.40	29.26	12.72	51.47	36.92
5	67	233.71	60.26	6.18	11.41	18.95	7.62	38.71	27.09

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The D^2 analysis for 36 accessions of sorghum indicated the presence of vast diversity and this can be further exploited by hybridization programmes. The traits such as grain yield per plant (g), days to flowering, panicle(s) weight per plant (g) contributed a higher proportion to total diversity. This study also identified promising accessions for higher yield and early maturity. Coherent selection of accessions in each cluster for economically important traits and combining different accessions with desirable trait means in different clusters can help in developing cultivars with multiple desirable traits in a broader genetic base.

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