Genetic diversity analysis in post rainy sorghum landraces and identification of rabi adaptive traits specific genotypes

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Abstract

Assessment of genetic diversity among sorghum landraces is essential for their efficient utilization in plant breeding. In the present study, 203 sorghum landrace accessions were phenotyped for two rabi growing seasons of 2016-17 and 2017-18 in a Randomized Block Design with three replications. Among the traits investigated, plant height was found more variable followed by dry fodder yield per plant, panicle weight per plant and grain yield per plant. The highest PCV and GCV was shown by dry fodder yield per plant (41.36 and 35.22 %), panicle weight per plant (38.49 and 32.84 %), grain yield per plant (36.30 and 27.95) and panicle length (33.06 and 29.81), respectively. The PCA revealed three important principal components correlated with grain yield per plant, panicle length and plant height that accounted for 70.30% of the total variation. The maximum inter-cluster distance was observed between cluster IV and V. Cluster VII had the highest mean value for grain and fodder yield.

Keywords: Cluster, landrace, phenotypic traits, principal component analysis, Sorghum.

1. Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth most important cereal crop worldwide after rice, wheat, maize and barley (Kholova *et al.*, 2013). It is used as source of food, feed, fodder, and biofuel. World over sorghum is cultivated in nearly 42.10 million ha with an annual production of 59.30 million tons (FAOSTAT, 2018). Among sorghum producing countries, India ranks first in terms of area but fourth to the USA, and for total production (FAOSTAT, 2018). India produces 4.80 million tons of sorghum from 4.96 million ha of land (FAOSTAT, 2018). In India, two distinct adaptive types – kharif (rainy) and rabi (post-rainy) sorghum are cultivated. The kharif sorghum is mainly used for non-food purposes, while *rabi* sorghum is entirely consumed as food grain (Patil *et al.*,

2013). Approximately 3.8 million tons of sorghum grain is produced from 4.6 million ha during the *rabi*. The stover obtained from rabi sorghum is highly valued as animal fodder (Blummel *et al.*, 2006). The loss of area under kharif sorghum is very high, while that of rabi sorghum is relatively stable. Rabi sorghum production is concentrated in southern Maharashtra, northern Karnataka and western Andhra Pradesh (Pray *et al.,* 2009). Rabi season is characterized by limited rainfall, cooler temperature (particularly night temperature) and shorter day lengths. *Rabi* sorghum predominantly is sown with late monsoon rains during end of August and September months.

Landraces are the locally adapted and preferred varieties nurtured and cultivated by the farmers through the traditional method of selection over the decades (Elangovan *et al.*, 2012). The need for further critical and systematic evaluation of these landraces and its utilization in grain and forage sorghum improvements is keenly felt. Thus the knowledge of genetic diversity among sorghum genotypes is essential for their efficient utilization in plant breeding programmes. Heritability of a trait is important in determining its response to selection and assists breeders to allocate resources necessary to effectively select for desired traits and to achieve maximum genetic gain with little time and resources (Al-Naggar *et al.,* 2018).

Over the years, a number of studies have dealt with estimating genetic diversity in cultivated sorghum using morphological traits (Zongo *et al.,* 1993; Appa *et al.*, 1996; Ayana *et al.*, 1998; Kumar *et al.*, 1999; Dahlberg *et al.*, 2002; Shehzad *et al.*, 2009; Adugna *et al.*, 2014). The use of morphological traits is the most common approach utilized to estimate relationships between genotypes. The genetic variability of cultivated species/varieties and their wild relatives together form a potential and continued source for breeding new and improved crop varieties. The availability of diverse and resilient genetic resources determines the current and future plant breeding and sustainability of agricultural productivity. Therefore there is a need to evaluate the available accessions for genetic diversity. In the present study, an attempt has been made to determine the extent of diversity among 203 sorghum landraces using the quantitative traits.

2. Materials and Methods

2.1 Plant materials

The material consists of 203 sorghum landraces originally collected from major rabi sorghum growing states of the country *viz.,* Maharashtra, Karnataka, Andhra Pradesh, Telangana, Tamil Nadu, etc. The seeds of these entries were obtained from the Gene banks of ICAR-Indian Institute of Millets Research, Hyderabad and the International Crops Research Institute for Semi-Arid Tropics (ICRISAT), Hyderabad (Table S1).

2.2 Study site, experimental design, data collection

Experiments were conducted at the experimental research farm of the Centre on Rabi Sorghum (ICAR- Indian Institute of Millets Research) located at 17° 40' N latitude and 75° 54' E longitude at an elevation of 476.5 meters above mean sea level (MSL) during two rabi seasons of 2016-17 and 2017-18. The average rainfall during the crop season was 125 mm, average temperatures (°C) ranged between 33.0 (max.) to 17.0 °C (min.) and Relative humidity (RH%) ranged between 61.28 (max) to 26.4 (min).

The experiments were laid out in Randomized Block Design with two replications. Each accession was raised in two rows of 3 meters length by adopting a spacing of 45 cm × 15 cm. In each replication, five random plants were chosen and the observations were recorded on these five plants for eight quantitative traits at the time of maturity except days to 50 percent flowering. Data were collected according to the Standard Key Descriptor Lists for Characterizations for sorghum (IIMR and ICRISAT 1984). The quantitative characters measured were days to 50% flowering (DF), plant height (PH) measured from the ground to the tip of the panicle at maturity (cm), panicle exertion (PE) measured from flag leaf to the base of panicle inflorescence (cm), panicle length (PL) measured from the lower panicle branch to the tip of the panicle at maturity (cm), panicle weight per plant (PW) measured as the weight of the panicle before threshing (g), grain yield per plant (GYPP) measured as the mean weight of the grains obtained from five panicles after threshing (g), fodder yield per plant (FYPP) measured as the mean weight of the dry fodder of the five plants (g) and 1000-seed weight (TSW) measured by weighing 1000 grains (g) at 12% moisture content. All the recommended agronomic packages of practices such as irrigation, fertilizer doses, and crop protection management were adopted during the entire crop period. The mean values were utilized for genetic diversity analyses among the rabi sorghum accessions.

2.3 Data Analysis

The descriptive statistics (mean, standard error, standard deviation, range & coefficient of variation), pooled analysis of variance (ANOVA), LSD, correlation & path analysis were computed for all the eight quantitative traits using R software (R: agricolae package version 1.3-2, 2020). Genetic diversity and cluster analysis were performed using R:stats version 3.6.2. Factor analysis was performed to know which trait contributed to maximum diversity. Principal component analysis of the traits was employed to examine the percentage contribution of each trait to total

genetic variation. PCA & factor analysis was done by using R: FactoMineR version 2.3 & R: agricolae version 1.3-2. Agglomerative hierarchical clustering was performed on the Euclidean distance matrix utilizing Ward's linkage method. R software packages of the latest versions were used for above analyses and representation.

3. Results and Discussion

3.1. Analysis of Variance (ANOVA)

The pooled analysis of variance over two similar rabi growing seasons using a randomized block design indicated significant variation for all 8 quantitative traits (Table S2). Genotype × Environment interaction was significant for the traits days to 50% flowering, plant height, panicle length, and 1000 seed weight, indicating the influence of the environment on the expression of these traits.

3.2. Descriptive and Genetic Statistics

Statistical analysis was carried out with the data on eight quantitative traits to assess the variability pattern (Table 1). Among all the traits investigated, plant height was found more variable with a mean value of 171.90 and range of 155.83, followed by dry fodder yield per plant (62.75 and 91.38), panicle weight per plant per plant (52.40 and 85.16) and grain yield per plant (37.74 and 52.73), respectively. The descriptive statistics of eight quantitative traits indicated the existence of good morphological diversity

and trait distribution among the sorghum landrace accessions, providing scope for improvement through hybridization and selection. The coefficient of variation for grain yield and dry fodder yield per plant were high indicating environmental fluctuations influencing their expression.

Phenotypic and genotypic variances as well as their coefficients of variation (GCV & PCV), heritability and expected genetic advance are presented in Table 1. In general, the estimates of phenotypic coefficient of variation (PCV) were higher than genotypic coefficient of variation (GCV). Combined across two environments, the highest PCV and GCV was shown by dry fodder yield per plant (41.36 and 35.22 %) followed by panicle weight per plant per plant (38.49 and 32.84 %), grain yield per plant (36.30 and 27.95) and panicle length (33.06 and 29.81), respectively, indicating that selection for high values of these traits of sorghum would be effective. Heritability ranged from 62% (days to 50% flowering) to 81% (panicle length), whereas genetic advance ranged from low (7.59) in panicle length to high (61.15) in plant height). The traits such as dry fodder yield per plant, panicle weight per plant and plant height showed high heritability coupled with high genetic advance, denoting there are good opportunities to get success in improvement of these traits via selection procedures.

Table 1. Statistics Summary for eight quantitative traits.

	DFF	PH (cm)	PE (cm)	PL (cm)	PW (cm)	GYPP (g)	FYPP (g)	TSW (g)
Descriptive Statistics								
Mean	67.02	171.90	32.33	13.71	52.40	37.74	62.75	28.98
Standard Error	0.40	1.33	0.38	0.14	0.73	0.61	0.95	0.27
Standard deviation	5.64	18.89	5.36	1.96	10.41	8.74	13.58	3.89
Range	30.25	155.83	35.45	19.60	85.16	52.73	91.38	23.45
Coefficient of Variation (%)	8.41	10.99	16.59	14.30	19.88	23.15	21.65	13.41
Genetic Statistics								
GV	52.45	1153.65	85.33	16.70	296.18	111.29	488.51	29.90
PV	84.25	1510.45	114.10	20.54	406.70	187.67	673.49	45.00
GCV	10.81	19.76	28.57	29.81	32.84	27.95	35.22	18.87
PCV	13.70	22.61	33.04	33.06	38.49	36.30	41.36	23.15
h^2 (%)	62.00	76.00	75.00	81.00	73.00	59.00	73.00	66.00
GA	11.77	61.15	16.46	7.59	30.25	16.73	38.78	9.18
GAM	17.56	35.57	50.90	55.37	57.74	44.34	61.80	31.68

DFF-Days to 50% flowering; PH-Plant height; PE-Panicle exertion, PL-Panicle length; PW-Panicle weight; GYPP-Grain yield per plant; FYPP-Fodder yield per plant; TSW-Thousand seed weight.

GV-Genotypic variance; PV-Phenotypic variance; GCV & PCV- Genotypic & Phenotypic coefficient of variation; h^2-Heritability; GA-Genetic advance; GAM-Genetic advance as percent of mean

3.3. Correlation and association between the traits

The correlation coefficients of eight quantitative traits were used in characterizing the 203 sorghum accessions. The correlation coefficients of eight quantitative traits estimated are presented in Figure 1. The high positive and significant correlation value was obtained for panicle weight per plant and dry fodder yield per plant with grain yield per plant. Grain yield per plant also showed a positive and significant correlation with days to flowering, plant height, panicle weight per plant, dry fodder yield per plant, and 1000 seed weight. The grain yield was also positively and significantly associated with days to 50% flowering, plant height, and 1000 seed weight. From these results, it is evident that these traits are associated with grain yield and are inter-correlated among them. Thus, the selection in any one of these yields attributing traits will lead to an increase in the other traits, thereby finally enhancing the grain and fodder yield, typically required for rabi genotypes. Hence, selection for traits like panicle weight per plant, and 1000-seed weight may also be given importance along with yield.
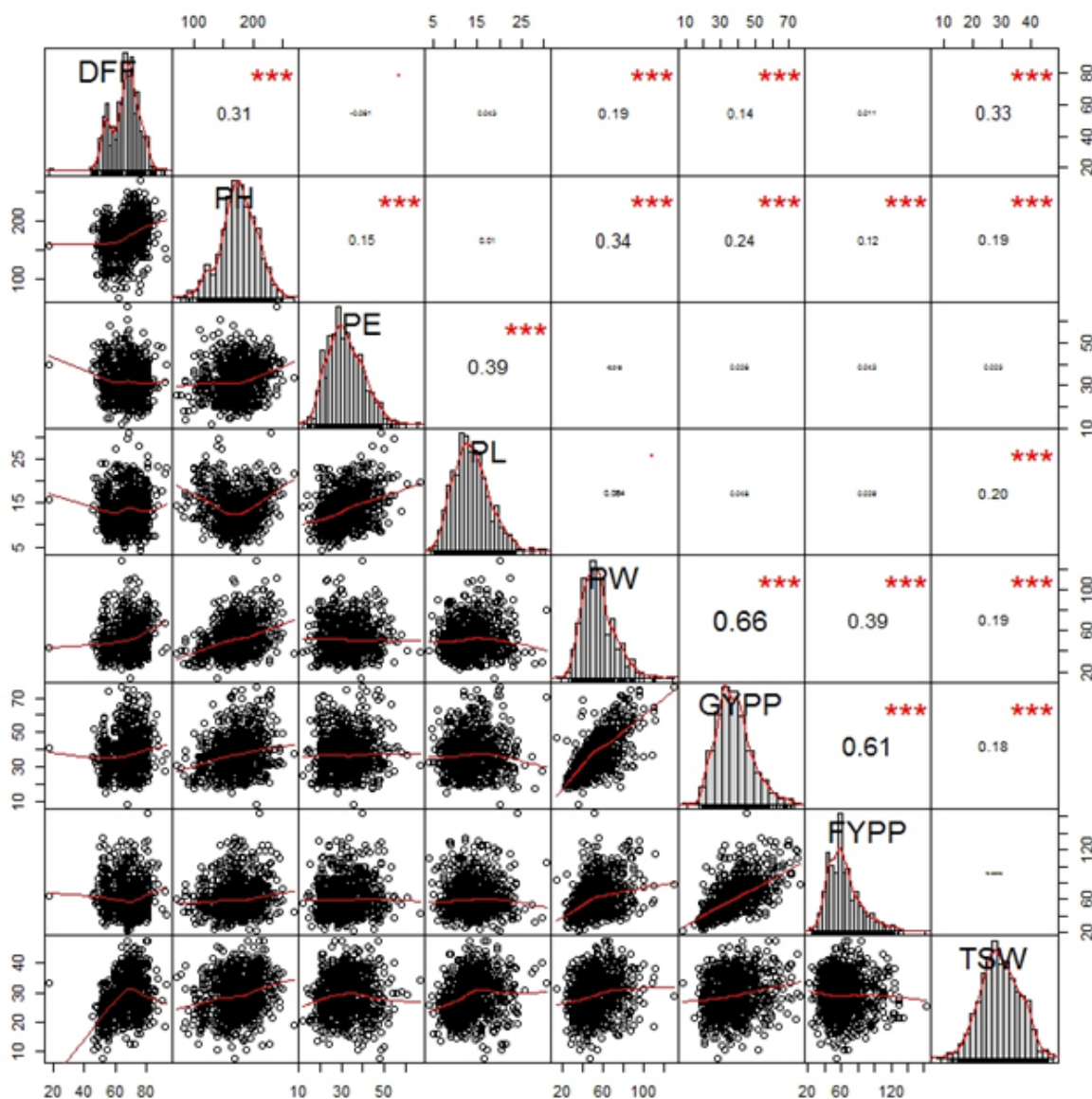

Figure 1. The pairwise correlations of different traits for sorghum germplasm accessions. The upper-right panels show the numerical values of the correlations (rounded to two decimal places). The* and ** indicate correlation significance at P 0.05 and P 0.01 levels of probability, respectively. The lower-left panels show the scatter plots. Abbreviations: DFF, days to 50% flowering; PH, plant height; PE, panicle exertion; PL, panicle length; PW, panicle weight per plant; GYPP, grain yield per plant; FYPP; dry fodder yield per plant; TSW, thousand seed weight.

Partitioning of yield and yield components into direct and indirect effects (result not shown) revealed that character panicle weight per plant (0.4765) exhibited highest positive direct effect on grain yield per plant followed by dry fodder yield per plant (0.4233) and 1000 seed weight (0.0867). The characters panicle weight per plant via dry fodder yield per plant (0.1651) followed by plant height via panicle weight per plant (0.162) and 1000 seed weight via panicle weight per plant (0.0905) showed indirect effect on grain yield per plant (Table S6).

3.4. Factor Analysis

Factor analysis was performed to reduce a large set of phenotypic traits to a more meaningful smaller set of traits and to know which trait is contributing to maximum variability. Thus, based on factor analysis, the quantitative traits that are contributing maximum variability to the first four factors are selected for principal component analysis (Table S3). The first four factors are contributing to 68.30 % of the total variance observed. The first factor had high contributing factor loading from grain yield per plant, panicle weight per plant/plant, and dry fodder yield per plant and contributed to 24.70% of the total variation. The second factor had high contributing loading from plant height, days to 50% flowering, panicle weight per plant, and thousand-seed weight and contributed to 17.80% of the total variation. The third factor had high contributing loading from panicle exertion, panicle length, and plant height and contributed to 13.40% of the total variation. The fourth factor had high contributing loading from panicle length and panicle exertion and contributed to

12.40% of the variation. The distribution of biometrical traits in the first two factors is shown in the loading plot. The loading plot clearly showed that the traits panicle weight per plant, grain yield per plant, dry fodder yield per plant, and 1000 seed weight had contributed low variability towards genetic variation.

3.5. Principal Component Analysis

All the eight quantitative traits were used to group the accessions based on the principal component. The first three principal components accounted for 70.3% of the total variance (Table 2). The first principal component (PC1) accounted for 34.81% of the total variance and had high contributing factor loading from grain yield per plant, panicle weight per plant, dry fodder yield per plant, plant height, days to 50% flowering stem and thousand-seed weight. The second principal component (PC2) had high contributing factor loading from panicle length, panicle exertion, dry fodder yield per plant, and grain yield per plant and contributed to 20.65% of the total variation. The third principal component (PC3) accounted for 14.84 % of the total variation, with high factor loading for plant height, panicle exertion, days to 50% flowering, panicle length and 1000 seed weight. The PCA analysis revealed that the grain yield per plant, dry fodder yield per plant, and panicle weight per plant contributed maximum towards divergence. The distribution pattern also indicated the existence of a significant amount of variability among the rabi sorghum landraces (Figure S1). The biplot of 203 accessions based on the first two principal components is presented in Figure S2.

Table 2. Principal components analysis showing the contribution of 8 characters among the sorghum accessions.

Variable	PC1	PC2	PC3
Days to 50% Flowering	0.592	-0.349	0.426
Plant Height	0.635	-0.13	0.574
Panicle Exertion	-0.066	0.754	0.494
Panicle Length	-0.151	0.792	0.243
Panicle weight per plant	0.837	0.117	-0.174
Grain yield per plant	0.856	0.258	-0.286
Dry fodder yield per plant	0.636	0.399	-0.426
1000 Seed weight	0.409	-0.278	0.281
Eigen value	2.785	1.652	1.187
% variance	34.81	20.651	14.839
Cumulative % variance	34.81	55.46	70.299

3.6. Cluster Analysis

Agglomerative hierarchical clustering performed on the Euclidean distance matrix utilizing Ward's linkage method and resulting dendrogram is presented in Figure 2. The 203 sorghum accessions formed ten clusters. Among the
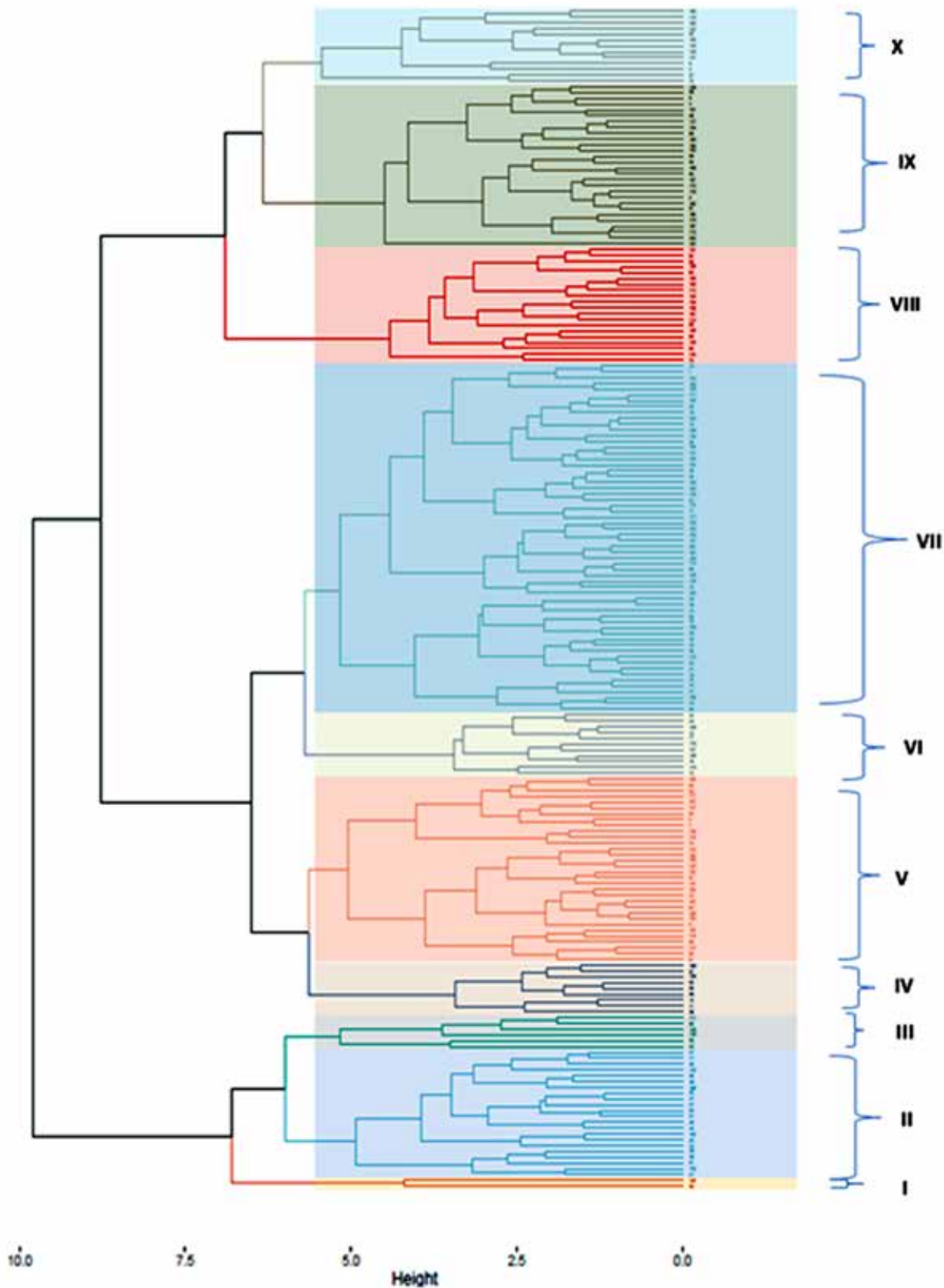

Figure 2: Dendrogram of sorghum accessions based on eight quantitative traits.

different clusters, the cluster size varied from 2 to 60. The maximum number of accessions was included in cluster VII having 60 accessions and the minimum number in cluster I having 2 accessions. Clusters along with the number of accessions and list of genotypes are presented in Table S5. The clustering pattern indicated the existence of a significant amount of variability among the sorghum landraces studied.

The highest inter-cluster distance was observed between cluster IV and V (157.12); the accessions from those clusters if chosen for hybridization program give a broad spectrum of variability in segregating generation (Table S4). The lowest inter-cluster distance was observed between VIII and X (56.82). The clusters contributing maximum to the divergence were given greater emphasis for deciding the type of cluster for further selection and the choice of the parents for hybridization. The cluster mean of the ten similarity cluster groups in the 203 sorghum accessions is presented in **Table 3.** Cluster VII had the highest mean values for days to 50% flowering (74.65), panicle exertion

(36.67), grain yield per plant (53.94) and dry fodder yield per plant (109.85). Cluster V showed the highest mean values for plant height (207.31) and 1000 seed weight (31.47). Cluster IV showed the highest mean value for panicle length (17.00), whereas cluster IX had the highest mean value for panicle weight per plant (76.08). And clusters such as V & IX showed moderate mean values for days to 50% flowering (73.45, 70.17), VII & III for plant height (200.75, 195.74), III & X for panicle exertion (36.56, 35.80), VII & V for panicle length (16.15, 15.30), VII & V for panicle weight per plant (75.95, 65.69), IX & I for grain yield per plant (48.55, 45.29), I & VI for dry fodder yield per plant (92.03, 79.38) and clusters II & VI for 1000 seed weight (30.55, 29.41). Based on the cluster means, the important cluster is cluster VII which had the highest mean values for panicle exertion, grain yield per plant, and dry fodder yield per plant. Hence the accessions falling under these clusters could be used as the parents for hybridization programs.

Table 3. Characteristic means of ten cluster groups of sorghum accessions

Trait	I	II	III	IV	V	VI	VII	VIII	IX	X
Days to 50% Flowering	62.39	69.90	67.06	62.29	73.45	67.05	74.65	66.01	70.17	62.44
Plant Height	153.84	179.44	195.74	121.67	207.31	184.77	200.75	164.19	172.86	162.18
Panicle Exertion	33.36	29.88	36.56	31.08	35.25	33.18	36.67	30.10	24.83	35.80
Panicle Length	13.07	11.82	14.51	17.00	15.30	13.28	16.15	12.03	12.73	13.85
Panicle weight per plant	53.89	54.46	43.20	42.04	65.69	55.47	75.95	40.50	76.08	50.32
Grain yield per plant	45.29	37.82	31.64	31.28	44.58	42.48	53.94	27.24	48.55	37.04
Dry fodder yield per plant	92.03	54.13	53.37	53.99	70.05	79.38	109.85	44.71	71.97	62.38
1000 seed weight	28.31	30.55	28.80	26.36	31.47	29.41	28.52	28.18	29.36	28.18

3.7. Identification of trait specific and promising genotypes:

203 landraces evaluated for two seasons were categorized into different trait-specific classes. The categorization is based on most suitable and desirable rabi adaptive trait measurements such as early to medium in duration (<60 days), medium-tall height (170-180 cm) - to avoid lodging, better panicle exertion (>40cm), medium to long panicle

size (>20cm), good panicle weight per plant/plant (>75g), superior grain yield per plant (>50g) and dry fodder yield per plant (>80g) along with moderate 1000 seed weight (>35.0g), as both grain and fodder are important under rabi situation. The trait-specific germplasm identified from the study is presented in Table 4.

Table 4. Trait specific promising genotypes identified from the study

Trait	Promising Genotypes
Early flowering (<60 days)	IS 18411, IS 18482, IS 18388, IS 22109, PVRL 16-1, IS 18437, IS 18030, IS 18347, PVRL 16-7, IS 18385, IS 23402, IS 18408, IS 2176, IS 23441, IS 18509, IS 4948, IS 18512, PVRL 16-5, IS 18344, PVRL 16-8, 3062-1
Medium Tall (170-180 cm)	TSLC-25, TSLC-26, TSLC-18, IS 18323, 3190, PVRL 16-6, RSLG2222, PVRL 16-12, IS 18385, IS 5221, IS 24365, RSLG 2389, RSLG 2386, 3178-3, IS 18347, IS 4895, IS 5544, IS 4597, PVRL 16-10, 3030, 3133, IS 24339, 3047, IS 17853, RSLG 2380, IS 17774, 3156, IS 22109, 3130-1, IS 5581, IS 18508, IS 5031, TSLC-21, TSLC-11, 3081-2, IS 17962, IS 18322, IS 5641, IS 26981, IS 5241
Better panicle exertion (>40 cm)	PVRL 16-5, IS 24329, IS 18028, WASHIM LOCAL-4, IS 18513, IS 5581, IS 18446, PVRL 16-8, 3070-2, IS 18657, IS 24345, RSLG 2387, 3001, IS 5586, IS 18344, IS 4959, RSLG 2231, IS 18437, IS 22127, IS 24338, IS 23441, IS 4940, WANI LOCAL, IS 23413, IS 24331, 3122-1
Panicle Length (>20 cm)	IS 18446, IS 18474, IS 18344, IS 23441, PVRL 16-8, IS 18486
Panicle weight per plant (>75 gm)	IS 4895, RSLG 2261, IS 17957, IS 5036, WASHIM LOCAL-4, IS 24361, CSV 29, RSLG 2386, 3125, WASHIM LOCAL-9, RSLG 2241, IS 17959
Grain yield per plant (>50 gm)	IS 4895, WASHIM LOCAL-4, WASHIM LOCAL-9, RSLG 2241, IS 24361, EKARJUNA-271, RSLG 2231, CSV 29, PVRL 16-1, TSLC-9, 3125, IS 22117, TSLC-4, IS 18385, RSLG 2380
Dry fodder yield per plant (>80 gm)	IS 22117, WASHIM LOCAL-4, RSLG 2387, IS 18322, IS 4506, IS 4948, 3128, WASHIM LOCAL-9, RSLG 2261, RSLG 2380, RSLG2222, IS 18385, IS 18495, RSLG 2218, IS 4958, RSLG 2238, IS 22152, IS 3815, IS 18672, IS 23441, TSLC-26, EKARJUNA-271, IS 18408, IS 2176, IS 18357, RSLG 2241, IS 22116, WARDHA LOCAL, PVRL 16-1, HINGANGHAT LOCAL, Phule Mauli
1000 Seed weight (>35 gm)	TSLC-27, TSLC-5, TSLC-25, TSLC-23, 3138-1, 3144-2, TSLC-1, TSLC-4, 3139-2, TSLC-19, IS 17909, RSLG 2382, IS 18396, IS 24365, TSLC-11

Plant Genetic Resources (PGR) contributes significantly towards food security, environmental protection, and sustainable development (Christine *et al.*, 2011). They represent a critical component of crop improvement efforts aimed at increasing food security both for short term gain and long term increase in productivity. In the present investigation, combined across two environments, the highest PCV and GCV was shown by dry fodder yield per plant, panicle weight per plant, grain yield per plant and panicle length, indicating that selection for high values of these traits of sorghum would be effective. The similar findings were also reported by Massaoudou *et al*, 2018. The traits such as dry fodder yield per plant, panicle weight per plant and plant height showed high heritability coupled with high genetic advance, indicating that there are good opportunities to get success in improvement of these traits via selection procedures (Arunkumar *et al.,* 2004). The correlation and association studies, indicated some important associations among the quantitative traits studied. The traits, namely, panicle weight per plant and

dry fodder yield per plant and 1000 seed weight had a significant and positive association with grain yield (Shubhanshu *et al.*, 2020). Partitioning of yield and yield components into direct and indirect effects revealed that character panicle weight per plant exhibited highest positive direct effect on grain yield per plant followed by dry fodder yield per plant and 1000 seed weight and these results are in accordance with Elangovan *et al*, 2012. The principle component analysis and hierarchical cluster analysis grouped the sorghum accessions under ten clusters. Hence, the selection of parents must be based on the wider inter-cluster distance and superior mean performance for yield and yield components. Application of the PCA tool and multivariate statistical analysis provide useful means to estimate morphological diversity within and between germplasm collections (Adugna, 2014; Maji *et al.,* 2012). In this study, three axes contributed 70.3% of total diversity among the accessions. Earlier reports also suggested the important contribution

of first PCs in total variability while studying different traits (Adugna, 2014; Maji *et al.,* 2012).

4. Conclusion

The studied accessions showed high variability for all the quantitative traits. The traits with high heritability coupled with high genetic advance, are further chosen for improvement via selection procedures. Pearson correlation coefficient analysis revealed a significant positive association between economic traits that can be used in the improvement of breeding activities in sorghum by breeders. The similarity cluster analysis facilitated the classification of the 203 accessions under ten cluster groups. Five accessions *viz.,* WASHIM LOCAL-4, IS 4895, WASHIM LOCAL-9, IS 24361, IS 22117 were found to be most potential (panicle weight per plant, grain and fodder yield) towards direct utilization in sorghum improvement. These may be further evaluated in multilocation trials. Some lines like PVRL 16-1, IS 18513, IS 18474, IS 17957, IS 17959, EKARJUNA-271 and IS 4948, identified in the study can be involved in crossing programs to create maximum diversity for the benefit of new varieties development.

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Author's contribution

Conceptualization of research (PP); Designing of the experiments (PP, RM, NSD); Contribution of experimental materials (PP, ME, MYS); Execution of field/lab experiments and data collection (PP, MYS, KKS, ARL); Analysis of data and interpretation (PP, RM, NSD); Preparation of the manuscript (PP, RM, KKS).

Declaration

The authors declare no conflict of interest.

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