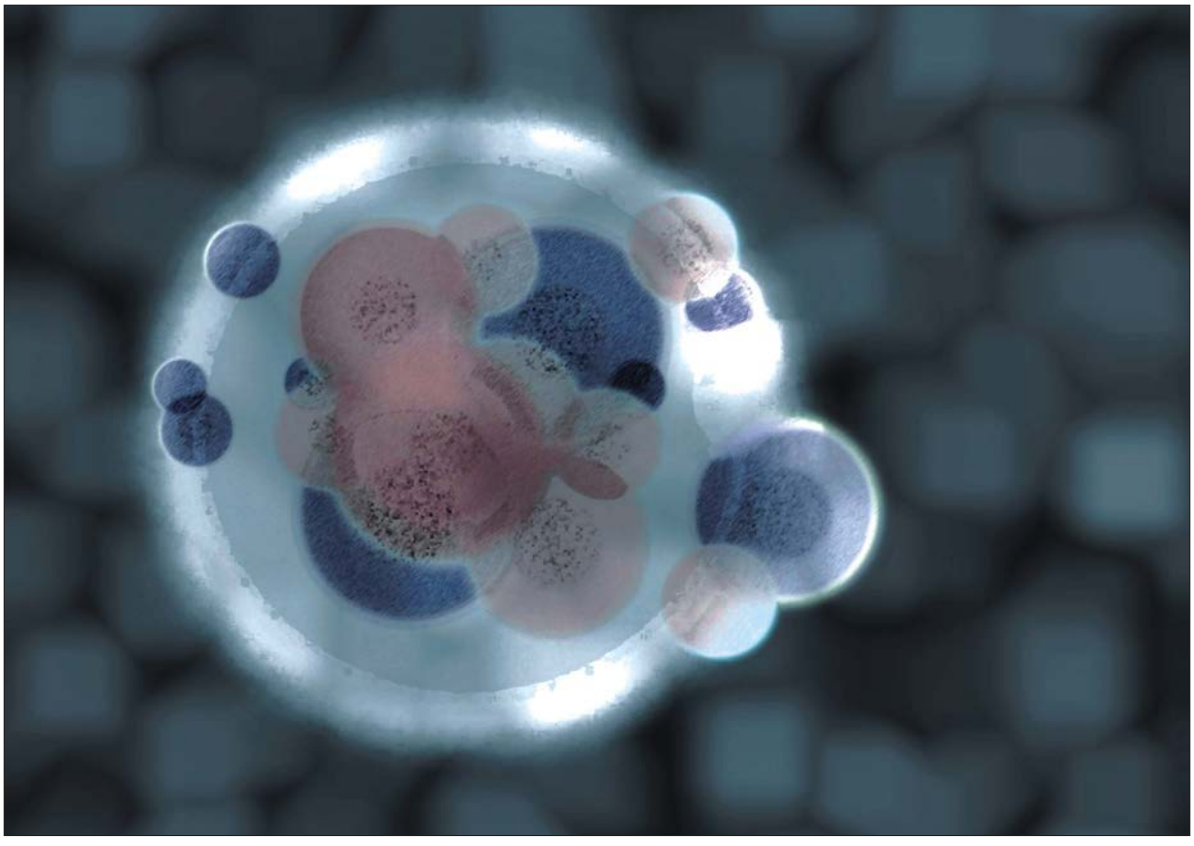

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Multivariate Analysis of Nutritional Diversity in Sorghum Landrace Accessions from Western Ethiopia

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Abstract: In Ethiopia, sorghum is grown for food and cash income by subsistence farmers. The study was conducted at the experimental farm of the Agricultural Research Council, Grain Crops Institute at Potchefstroom, South Africa. A total of 31 sorghum landrace accessions were used for chemical analysis. The objective of this study was to determine the extent of genetic diversity in nutritional composition of sorghum landraces from western Ethiopia. Sorghum whole grains were analyzed for crude protein, total starch and its component and mineral profile (calcium, magnesium, potassium, phosphorus, iron, manganese, zinc and sodium). The Principal Component Analysis (PCA) revealed that the first four principal components contributed 71.77% of the variability among sorghum landrace accessions. Mineral elements such as zinc, manganese, magnesium, phosphorus and protein contributed more divergence to the first Principal Component (PC1), while iron, sodium and calcium contributed to the second Principal Component (PC2). Cluster analysis of mineral elements, protein, total starch and sugar contents resulted in five distinct groups of accessions with genetic distances ranging from 0.78-1.52. Therefore, the chemical compositions provide a useful measure of genetic divergence among sorghum landrace accessions to identify potential donors or parental lines for future sorghum quality improvement effort.

Key words: Genetic diversity, landrace, nutritional composition, principal component analysis, sorghum accessions

INTRODUCTION

Sorghum is an indigenous and second most important cereal crop in Africa (Elkhalifa and El-Tinay, 2002). It shows a higher tolerance to drought, that makes it a crop of preference in the tropical and subtropical areas in the world (Hulse *et al*., 1980; Gualtieri and Rappaccini, 1990). In developing countries like Ethiopia, sorghum can play an important role in achieving food security at the household level (Dendy, 1995) and generate income. A large number of different sorghum landrace accessions, which are adapted to different environmental factors, are cultivated by growers in different parts of Ethiopia.

With respect to sorghum improvement programmes in the country, the conservation and utilization of genetic resources is incredibly important, since sorghum landraces provide useful genes/alleles in terms of nutritional quality traits (Tanksley and McCouch, 1997). It is a rich source of mineral elements, vitamins, protein and carbohyderate which are important for human consumption. Cereals like sorghum have a potential for health enhancement and that their consumption can lower malnutrition (Topping, 2007) in Ethiopia.

Knowledge of genetic and nutritional diversity has an important impact on the improvement of sorghum for quality breeding as well as on the conservation of sorghum germplasm resource (Dean *et al*., 1999; Simioniuc *et al*., 2002) in Ethiopia.

Determination of nutritional diversity in sorghum accessions is incredibly important for improving malnutrition due to mineral elements, protein, starch and vitamins in food stuff (Peters *et al*., 2003; Welch and Graham, 2004; Feil *et al*., 2005). Quality assessment and breeding for higher concentration of mineral elements,

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protein, vitamins, total starch and sugar contents in sorghum is important for improving the health of humans (Gualtieri and Rappaccini, 1990).

Multivariate analysis has been found to be a powerful tool for quality assessment and to estimate the extent of genetic diversity for choosing potential parents for crossing blocks among the germplasm accessions in the breeding programme (Dasgupta and Das, 1984; Falcinelli *et al*., 1988; Chozin, 2007). Selection of sorghum accessions for improved nutritional characteristics is dependent on knowledge of the extent of genetic diversity expressed among them. Hence, the objective of the present study was to assess the extent of genetic diversity and interrelationship among sorghum landrace accessions from Western Ethiopia for nutritional composition using multivariate analysis.

MATERIALS AND METHODS

Plant material: All 31 sorghum landrace accessions used in this study were received from the Institute of Biodiversity Conservation/Ethiopia (Table 1).

Mineral content determination: Sorghum kernels were ground to a fine powder with an IKA Analysis A10 Grinder. Two gram flour samples were weighed into porcelain crucibles and oven dried in a muffle furnace at 550°C for 3 h. The samples were then acid-digested by addition of 1 mL of 55% (v/v) HNO_3. The acid was evaporated to dry from the samples using a sand bath and oven dried in the muffle furnace again. Ten mililiters of 1:2 v/v 55% HNO_3 solution was added to the samples to moisten them and placed in the sand bath for 5-10 min to warm it up. The samples were stirred in porcelain crucibles using glass rods and transferred into 100 mL volumetric flasks. The samples were shaken immediately and allowed to dissolve overnight to extract the minerals. The samples were then transferred into glass test tubes and diluted with distilled water 100 times. Mineral concentrations were then determined by an Atomic Absorption Spectrophotometer (SpectrAA 300).

Table 1: List of sorghum accessions used for study

No.	Accessions	No.	Accessions	No.	Accessions
1	69029	12	223548	23	229831
2	69030	13	223551	24	229832
3	69032	14	223552	25	229833
4	69127	15	223554	26	229834
5	69128	16	223555	27	229835
6	69147	17	223558	28	229838
7	69164	18	228736	29	237762
8	69165	19	228739	30	237763
9	69538	20	228740	31	237779
10	223525	21	228741		
11	223543	22	228919		

Protein content determination: Flour sample (250 mg) was weighed, oven dried over night at 95°C and protein content (Nitrogen×6.25) was determined by the combustion method (Leco ®model, FP-528, St. Joseph, Ml) in the Nutritional Laboratory, Department of Animal, Wild and Grassland Sciences, University of the Free State.

Starch extraction: Starch content was determined using a total starch assay procedure [Amyloglucosidase/α-Amylase Method (Megazyme International Ireland Ltd, Bray, Ireland)].

Amylose content determination: Amylose was extracted and estimated by the iodine binding method (Cruz and Khush, 2000). One hundred milligram of sorghum flour sample was weighed. The samples were wetted with addition of 1 mL of 95% (v/v) ethanol followed by 9 mL of 1 M NaOH to aid dispersion and stirred using a vortex mixer. The samples were placed in a boiling water bath for 15 min and stirred using a vortex mixer every 5 min. The samples were cooled at room temperature for 1 h and then centrifuged at 3000 rpm for 5 min. Duplicate 0.1 mL aliquots of the solution were transferred into clean test tubes and 0.1 mL of 1 M acetic acid was added to each test tube followed by addition of 0.2 mL iodine solution and 9.6 mL distilled water. The contents were vortexed and left to stand for 20 min. The absorbance was read against the reagent blank at 620 nm for each sample. The amylose percentage was calculated using the formula:

$$\text{Amylose (\%)} = \frac{\text{Concentration (mg mL}^{-1}) \times 1000}{\text{Mass of the sample (mg)}} \times 100$$

Sugar content determination: The total sugar content in stalks at physiological maturity was estimated as the Brix% using a hand held refractometer. The refractometer was calibrated with distilled water and the sugar content was measured. The samples were taken from the third internode from the base of the plant for uniformity of sampling. The sap was squeezed and extracted from the cut stalks with pliers and placed on a hand held refractometer, after which readings were taken.

Statistical data analysis: Multivariate analysis was employed using the appropriate procedure of the Number Cruncher Statistical System (Hintze, 2004). Principal Component Analysis (PCA) was used as a data reduction tool to summarise the information from chemical composition. Means of each variable were standardised prior to PCA as suggested by Ruiz *et al*. (1997). Standardisation is achieved by subtracting from each observation the mean value of the character and

subsequently dividing it by its respective standard deviation (Ruiz *et al*., 1997; Upadhyaya *et al*., 2002). The measure of genetic dissimilarity was Euclidean distance and the hierarchical agglomerative clustering method using the Unweighted Pair-Group Method with Arithmetic Mean (UPGMA) was used to examine the clustering of germplasm accessions. Euclidean measure of distance was also used for estimating Genetic Distance (GD) among accessions as described by Mohammadi and Prasanna (2003).

RESULTS AND DISCUSSION

The genetic diversity in 31 germplasm accessions was observed for the chemical compositions which are a pre requisite for the selection of parental lines in the future quality breeding programme. The PCA grouped the 11 chemical traits into 11 components, which accounted for the entire (100%) variability among the studied accessions. As Chatfield and Collins (1980) stated, components with an eigenvalue of less than 1 should be eliminated so that fewer components are dealt with. Furthermore, Hair *et al*. (1998) suggested that eigenvalues greater than one are considered significant and component loadings greater than ±0.3 were considered to be meaningful. Hence, from this study, only the first four eigenvectors which had eigenvalues greater than one and cumulatively explained about 71.77% of the total variation among the 11 chemical compositions describing the accessions (Table 2). Hence, PC1 had an eigenvalue of 3.82 and accounted for 34.76% of the variation. This represents an equivalent of five variables and indicated that Zn, Mn, Mg, P and protein were important contributing variables for the variations among the accessions. Accessions with high PC1 scores, therefore, would have high levels of these mineral elements and protein contents. The PC2 had an eigenvalue of 1.56, contributing 14.16% of the variation and had Fe concentration as the main contributing factor compared to sodium, calcium and protein content. Third Principal Component (PC3) had eigenvalues of 1.38, indicating that Mn, K and Na were contributing 12.51% variation. The fourth Principal Component (PC4) with 10.33 of the variance was composed of Ca, total starch and K, while sugar content concentration contributed a lesser amount. Protein concentration was important in at least two PCS, indicating its relative importance to variation among the accessions. The PC1 and PC2 explained most of the variation among the accessions. Similarly, Buffo *et al*. (1998) found the genetic variation among 46 commercial sorghum hybrids for quality factors using multivariate analysis. Similarly, Mweta (2009) reported the results of the Principal Component Analysis (PCA) and biplot performed on the chemical composition of the cocoyam, sweetpotato and cassava starches.

The biplot demarcated the accessions with chemical characteristics explained by the first two dimensions. A breeder, in consequence, can easily visualise the distances between the accessions and decide on the best varieties to be selected, based on several variables, compressed in the two major principal components and analysed simultaneously. Accessions close to each other in the scores plot are similar; accessions located near the origin are distinctive accessions and those far from the origin are extremes/distinct. This is because the principal component has been constructed with the data centred by subtracting the average of each variable. The PCA analysis grouped the accessions into groups over the four quadrants based on the concentrations of mineral elements, protein, total starch and sugar contents (Fig. 1). The accessions remained scattered in all four quadrants, showing large genetic variability in nutritional compositions. The accessions in the top left quadrant were closely related in Na and total sugar contents. The right top quadrant consisted of the accessions with related contents of Ca, Fe, Mn, Zn, protein and total starch. The right bottom quadrant comprised the accessions associated with the P, K and Mg on the first principal component. The distance between the locations of any two accessions on the score plot is directly proportional to the degree of difference/similarity between them in terms of the mineral and protein contents. Figure 2 therefore, revealed that accessions 69147 (#6), 223525 (#10), 223548 (#12), 223555 (#16), 223558 (#17), 237763 (#30) and 237779 (#31) were the most distant/diverging from the major group which in the principal component axes was concentrated on zero depicting some similarity in terms of the nutritional values.

Table 2: Principal Components (PCS) analysis of protein, total starch, sugar content and eight mineral elements in sorghum accessions showing eigenvectors, eigenvalues and their percentage contribution to the total variations explained with the first four principal component axes

		Total variance		Eigen vectors (loading) for										
PC	Eigenvalue	Individual (%)	Cumulative (%)	Zn	Fe	Mn	Ca	Mg	K	Na	P	Protein	Starch	Sugar
1	3.82	34.76	34.76	0.39	0.12	0.35	0.18	0.42	0.17	-0.14	0.43	0.39	0.14	-0.30
2	1.56	14.16	48.92	0.07	0.61	0.04	0.30	-0.25	-0.23	0.43	-0.09	0.30	0.25	0.26
3	1.38	12.51	61.43	-0.23	0.25	0.34	0.24	0.10	-0.53	-0.54	-0.13	-0.08	-0.32	0.01
4	1.14	10.33	71.77	0.25	0.16	-0.22	0.39	-0.02	0.44	-0.09	0.09	-0.10	-0.59	0.36

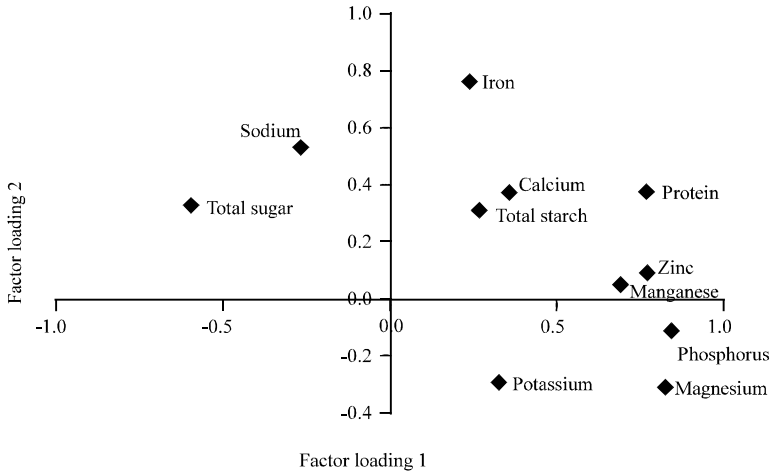

Fig. 1: Principal component analysis loading plot for mineral elements, total starch and total sugar contents of the sorghum landrace accessions

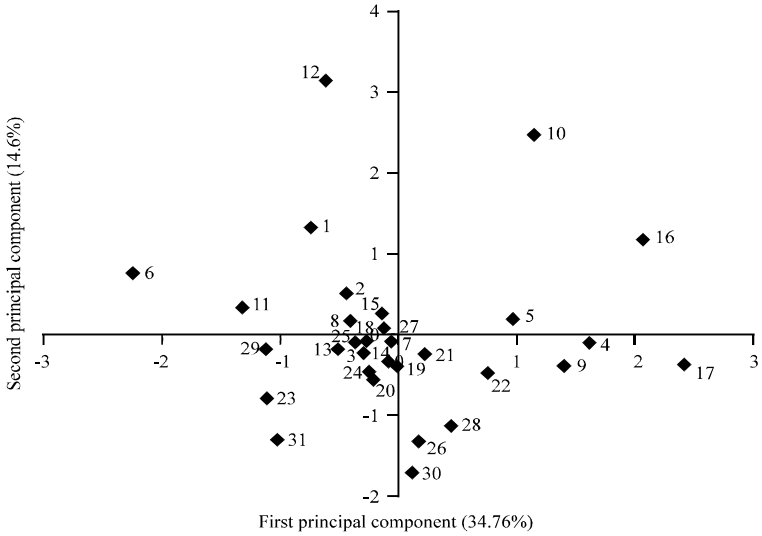

Fig. 2: Principal component analysis score plot of first and second principal components describing the overall variation among nutritional compositions from 31 sorghum landrace accessions; Accessions number as given in Table 1

However, accessions 223548 (# 12) showed a similar relationship in the second principal component axis with accession 237763 (# 30). Furthermore, accessions 223558 (# 17) and 223555 (# 16) revealed a close relationship in the second principal component axis. Accessions which overlapped in the principal component axes had similar relationships in the concentration of the mineral elements, starch, sugar and protein content. The loading plot indicates the similarities/correlations and differences between the chemical compositions. The elements with small loadings located near the origin have little influence on data structure; whereas, the elements, starch, sugar and protein with high loadings represent the greatest influence on data structure on the clustering and separation of sorghum accessions.

Genetic distance and cluster analysis: Estimates of genetic distances matrix based on nutritional traits for all pair-wise combinations of (31×30)/2 = 465 for the 31 sorghum landrace accessions are presented in Table 3. Genetic distances from 0.78-1.52 were observed in the pair-wise combinations, indicating that the accessions were diverse for the chemical composition of the traits measured. The minimum genetic distance of 0.78 and 0.79 were recorded between accessions 237763 and 229838 and between accessions 228739 as well as accession 228736,

Table 3: Estimates of genetic distance based on nutritional composition for all pair-wise comparison of 31 sorghum landrace accessions

	Accessions	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	69029	1.00																													
2	69030	1.40	1.00																												
3	69032	1.40	0.98	1.00																											
4	69127	1.40	1.31	1.31	1.00																										
5	69128	1.40	1.31	1.31	0.92	1.00																									
6	69147	1.52	1.52	1.52	1.52	1.52	1.00																								
7	69164	1.40	1.00	0.96	1.31	1.31	1.52	1.00																							
8	69165	1.40	1.05	1.05	1.31	1.31	1.52	1.05	1.00																						
9	69538	1.40	1.31	1.31	0.88	0.92	1.52	1.31	1.31	1.00																					
10	223525	1.40	1.31	1.31	1.27	1.27	1.52	1.31	1.31	1.27	1.00																				
11	223543	1.40	1.12	1.12	1.31	1.31	1.52	1.12	1.12	1.31	1.31	1.00																			
12	223548	1.38	1.40	1.40	1.40	1.31	1.52	1.40	1.40	1.40	1.40	1.40	1.00																		
13	223551	1.40	1.00	0.79	1.31	1.31	1.52	0.96	1.05	1.31	1.31	1.12	1.40	1.00																	
14	223552	1.40	1.05	1.05	1.31	1.31	1.52	1.05	0.88	1.31	1.31	1.12	1.40	1.05	1.00																
15	223554	1.40	1.05	1.05	1.31	1.31	1.52	1.05	0.98	1.31	1.31	1.12	1.40	1.05	0.98	1.00															
16	223555	1.40	1.31	1.31	1.14	1.14	1.52	1.31	1.31	1.14	1.27	1.31	1.40	1.31	1.31	1.31	1.00														
17	223558	1.40	1.31	1.31	1.14	1.14	1.52	1.31	1.31	1.14	1.27	1.31	1.40	1.31	1.31	1.31	0.96	1.00													
18	228736	1.40	1.05	1.05	1.31	1.31	1.52	1.05	0.85	1.31	1.31	1.12	1.40	1.05	0.88	0.98	1.31	1.31	1.00												
19	228739	1.40	1.05	1.05	1.31	1.31	1.52	1.05	0.85	1.31	1.31	1.12	1.40	1.05	0.88	0.98	1.31	1.31	0.79	1.00											
20	228740	1.40	1.05	1.05	1.31	1.31	1.52	1.05	0.98	1.31	1.31	1.12	1.40	1.05	0.98	0.96	1.31	1.31	0.98	0.98	1.00										
21	228741	1.40	1.00	0.96	1.31	1.31	1.52	0.84	1.05	1.31	1.31	1.12	1.40	0.96	1.05	1.05	1.31	1.31	1.05	1.05	1.05	1.00									
22	228919	1.40	1.31	1.31	0.96	0.96	1.52	1.31	1.31	0.96	1.27	1.31	1.40	1.31	1.31	1.31	1.14	1.14	1.31	1.31	1.31	1.31	1.00								
23	229831	1.40	1.09	1.09	1.31	1.31	1.52	1.09	1.09	1.31	1.31	1.12	1.40	1.09	1.09	1.09	1.31	1.31	1.09	1.09	1.09	1.09	1.31	1.00							
24	229832	1.40	1.12	1.12	1.31	1.31	1.52	1.12	1.12	1.31	1.31	1.00	1.40	1.12	1.12	1.12	1.31	1.31	1.12	1.12	1.12	1.12	1.31	1.12	1.00						
25	229833	1.40	1.00	0.96	1.31	1.31	1.52	0.80	1.05	1.31	1.31	1.12	1.40	0.96	1.05	1.05	1.31	1.31	1.05	1.05	1.05	0.84	1.31	1.09	1.12	1.00					
26	229834	1.40	1.05	1.05	1.31	1.31	1.52	1.05	0.92	1.31	1.31	1.12	1.40	1.05	0.92	0.98	1.31	1.31	0.92	0.92	0.98	1.05	1.31	1.09	1.12	1.05	1.00				
27	229835	1.40	1.05	1.05	1.31	1.31	1.52	1.05	0.89	1.31	1.31	1.12	1.40	1.05	0.89	0.98	1.31	1.31	0.89	0.89	0.98	1.05	1.31	1.09	1.12	1.05	0.92	1.00			
28	229838	1.40	1.05	1.05	1.31	1.31	1.52	1.05	0.92	1.31	1.31	1.12	1.40	1.05	0.92	0.98	1.31	1.31	0.92	0.92	0.98	1.05	1.31	1.09	1.12	1.05	0.84	0.92	1.00		
29	237762	1.40	1.09	1.09	1.31	1.31	1.52	1.09	1.09	1.31	1.31	1.12	1.40	1.09	1.09	1.09	1.31	1.31	1.09	1.09	1.09	1.09	1.31	0.94	1.12	1.09	1.09	1.09	1.09	1.00	
30	237763	1.40	1.05	1.05	1.31	1.31	1.52	1.05	0.92	1.31	1.31	1.12	1.40	1.05	0.92	0.98	1.31	1.31	0.92	0.92	0.98	1.05	1.31	1.09	1.12	1.05	0.84	0.92	0.78	1.09	1.00
31	237779	1.40	1.09	1.09	1.31	1.31	1.52	1.09	1.09	1.31	1.31	1.12	1.40	1.09	1.09	1.09	1.31	1.31	1.09	1.09	1.09	1.09	1.31	1.02	1.12	1.09	1.09	1.09	1.09	1.02	1.09

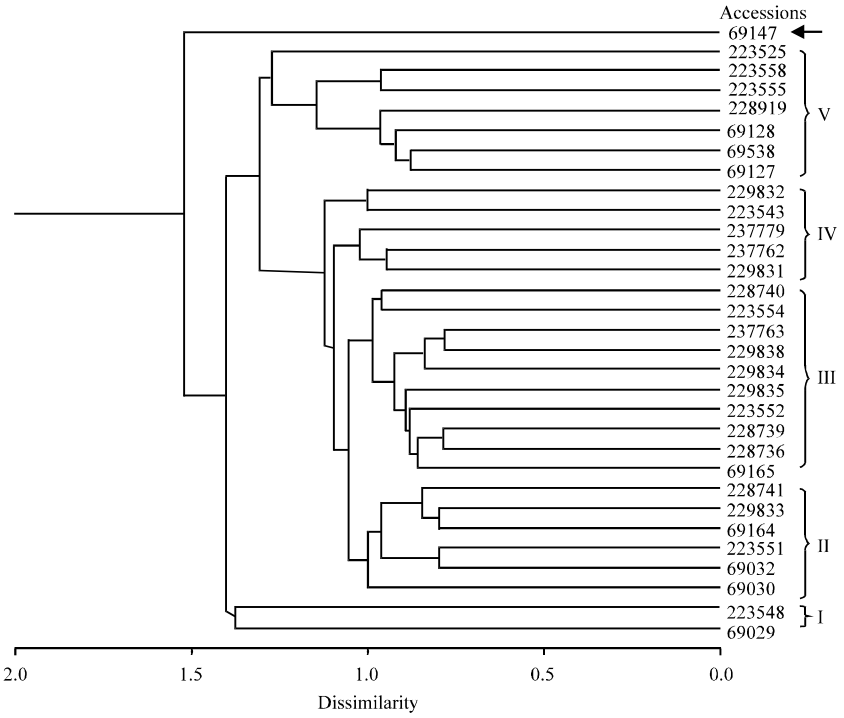

Fig. 3: Dendrogram of 31 sorghum landrace accessions revealed by UPGMA cluster analysis based on chemical composition data set

Table 4: Summary of cluster means of chemical compositions for the sorghum accessions based on data set

	Cluster means					
Characters	I	II	III	IV	V	Mean
Zn	26.58	22.42	23.81	21.03	30.00	24.77
Fe	92.00	50.86	58.62	49.79	71.99	57.82
Mn	13.83	12.96	16.43	15.52	19.33	15.61
Ca	288.67	310.96	273.33	284.50	389.11	309.31
Mg	975.00	1060.4	1257.21	1008.84	1357.13	1131.72
K	1784.38	1936.2	1626.06	1985.63	2032.15	1872.88
Na	43.94	23.70	19.22	21.08	20.38	25.66
P	2581.94	2917.1	3027.28	2269.15	3412.22	2841.54
Protein	10.60	10.88	10.26	9.40	12.85	10.80
Total starch	50.89	52.92	42.29	42.09	48.36	47.31
Sugar content	13.32	9.66	10.15	12.30	7.95	10.68
Mean	534.65	582.55	578.61	519.94	672.86	577.10

respectively. On the other hand, the highest genetic distance of 1.52 was recorded between accession 69147 and accessions 69029, 69030, 69032, 69127 and 69128 and the rest of the accessions, indicating that there was a high genetic diversity between the accessions due to chemical characteristics. Within the accessions there were 4 genetic distance values of lower than 0.80 indicating some relatedness within the germplasm accessions. This whole data set confirms that sufficient genetic diversity is present for the measured mineral elements, protein, total starch and sugar contents, although there is some relatedness. The accessions with the highest genetic distances between them can be included in crossing blocks of quality breeding programmes.

Cluster analysis for chemical composition shows a clear demarcation between sorghum germplasm accessions (Fig. 3). Furthermore, Table 4 showed differences among clusters by summarsing cluster means for the 11 biochemical traits. The highest cluster means was recorded in phosphorus compared to the rest of chemical traits, while the lowest was recorded in manganese, protein and total sugar content. Maximum cluster means was observed in cluster II (582.55) and cluster IV (672.86). This indicated the existence of maximum genetic divergence among germplasm accessions. Based on these chemical traits therefore, the accessions were grouped into different clusters. The dendrogram divided the accessions into five main clusters and a singleton. The first main cluster was produced at a genetic distance of 1.38 included the accessions 223548 and 69029. Cluster I characterised by the highest concentration of Fe, Na and total sugar content and lowest concentration of Mg (Table 4). The second main cluster was also formed at a genetic distance of 1.00 and comprised of six accessions. Accession 69030 was separated as a singleton accession from the rest in this cluster. This cluster consisted of accessions with the lowest Mn and total sugar content, but with the highest

total starch and average protein content. The third cluster consisted of ten accessions. This cluster grouped the accessions with average Fe and Mn content. It also grouped the accessions with the lowest concentration of Ca, K, Na and starch content. Accessions 228740 and 223554 were separated from this cluster due to the lowest concentration of Zn and the highest contents of Mn and total starch (Shegro *et al*., 2012). Cluster IV contained only five accessions namely, accessions 229832, 223543, 237779, 237762 and 29831 and formed at a genetic distance of about 1.00. This cluster consisted of an accession with lowest Zn, Fe, P, protein and total starch contents and average Mn concentration. Cluster V consists of seven accessions which were grouped based on the highest concentrations of Zn, Mn, Ca, Mg, K, P, protein and average total starch content, while the lowest total sugar content was also determined in this group. Accession 223525 was separated from this cluster due to the highest concentration of Fe (Shegro *et al*., 2012). Accession 69147 was not included in any of the clusters and grouped as a singleton and stood individually as a separate cluster and this indicates that it was dissimilar from the other accessions in regards of nutritional composition. Accession 69147 revealed the highest genetic dissimilarity coefficient value of 1.52 and appeared as the most divergent accession. This indicated that the accessions included in this study could be valuable sources of genetic variability in the sorghum improvement programmes for chemical composition. Therefore, the present study showed that the mineral, protein, total starch and sugar contents could classify the accessions according to their genetic similarity/differences by using multivariate analysis. Hence, selection and crossing of sorghum germplasm accessions included in different clusters would provide higher heterotic groups in sorghum breeding programme in Ethiopia and elsewhere. Similarly, Hasanuzzaman *et al.* (2002) found the genetic diversity among 33 sorghum indigenous cultivars of sorghum in Bangladesh using multivariate technique. Dasgupta and Das (1984) as well as Aremu *et al.* (2007) similarly reported the genetic diversity analysis using multivariate analysis for the selection of parents for hybridization.

Characterization of accessions and clustering them on the basis of their chemical traits will help in identification and selection of the best parents for hybridization (Souza and Sorrells, 1991) for the desirable traits. Similarly, Shegro (2010) found genetic divergence among sorghum germplasm accessions on the basis of their chemical composition and phenotypic traits using multivariate analysis tools. Moreover, Jamali *et al.* (2008) found the uptake of mineral elements using multivariate method of analysis using different sorghum varieties. Furthermore, they found that individual contents of elements seem to be appropriate descriptors for identifying suitable variety of sorghum. Mohammadi and Prasanna (2003) similarly found the multivariate analysis of population would group and assign the individuals into their appropriate population group. Shegro (2010) also reported the grouping of accessions by multivariate methods of analysis based on their similarity and divergences among the sorghum accessions. Therefore, the present study would be valuable for sorghum breeders in that the most important accessions in different clusters may be crossed with the accessions of the rest of the groups for improving the traits of interest.

CONCLUSION

Understanding the genetic diversity of sorghum germplasm collections is essential for effective exploitation of their genetic potential as well as for selection of landraces and other genotypes as breeding lines, maintenance and for conservation. The multivariate analysis technique has successfully provided estimation of genetic diversity among tested sorghum accessions based on chemical compositions. In sorghum therefore, quality improvement programme is necessary to critically identify and quantify germplasm accessions to improve their nutritional quality like mineral concentration, protein, total starch and sugar contents.

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