academicJournals

Vol. 9(5), pp. 314-321, May, 2015
DOI: 10.5897/AJFS2014.1284
Article Number: A63971453082
ISSN 1996-0794

http://www.academicjournals.org/AJFS

African Journal of Food Science

Full Length Research Paper

Genetic variation of biochemical characteristics of selected sorghum varieties from East Africa

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Received 16 February, 2015; Accepted 12 May, 2015

A total of 30 sorghum varieties from East Africa were analysed for their biochemical characteristics. The objective was to ascertain the extent of the genetic diversity underlying their biochemical and physiological characteristics that included starch (%), amylose (%), amylopectin (%), proteins (%), tannins (mg/100 ml), yield (Kg/ha) and height (cm). The principal component analysis (PCA) showed that the first two contributed to the 69.66% of the variability among the sorghum varieties. Cluster analysis of these parameters resulted into four distinct groups with a genetic distance ranging from 0.74 - 6.42. The open pollinated and the hybrids showed the greatest genetic distances while the hybrids exhibited relatively low genetic distances. The biochemical content is a useful tool for measuring the genetic divergence among sorghum varieties to identify possible donors for future sorghum quality enhancement/breeding.

Key words: Biochemical characteristics, principal component analysis, genetic distance, cluster analysis, sorghum breeding.

INTRODUCTION

Sorghum is one of the world's most important cereals, with over 500 million people in the hot dry tropics dependent on it. Approximately 35% of sorghum is utilised as a food grain and the balance is used primarily in alcohol production, animal feed and industrial products (Dicko et al., 2006; Mehmood et al., 2008). Sorghum requires less moisture than other cereal crops and is more tolerant to extreme environment, making production easier in most agro-ecological zones subject to limited rainfall areas which are unfavorable for most cereals (Maunder, 2002).

Evaluating genetic diversity of germplasm can assist in differentiating varieties with the greatest novelty which as a result is most desirable for incorporation into crop improvement programmes. Given the diversity of sorghum, studying genetic diversity (Ayana, 2001) and

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biochemical composition of sorghum germplasm from East Africa is very important for numerous reasons. Sorghum breeding programmes have offered a wide range of new varieties with interesting traits that improved production and productivity (FAO and ICRISAT, 1996). Screening and selection of improved varieties for specific local food and industrial requirements from the broad sorghum biodiversity is extremely important for food security and poverty alleviation (Akintayo and Sedgo, 2001; Dicko et al., 2006).

The variation of heritable characteristics present among alleles of genes in different individuals of populations of species serves as an important role in evolution that allows a species to adapt to a new environment is termed as genetic diversity (IPGRI, 1993; Weir, 1996; Kremer et al., 1998). Genetic distance estimates determined by phenotypic and molecular markers help identify suitable germplasm for incorporation into future plant breeding programmes. Hence, assessment of genetic diversity in sorghum germplasm and determination of sorghum phenotypic and biochemical activities would help to determine the breeding potential of the accessions in East Africa.

Quantitative assessment of genetic diversity is important in determining the extent of genetic difference between and within crop species (Adugna, 2002). Genetic distance is a measure of the average genetic divergence between two sequences, species or between populations within a species or taxa (Souza and Sorrells, 1991). Genetic similarity is the converse of genetic distances, that is, the extent of sequence similarities among cultivars.

The measure of distance or similarity among cultivars is the covariance of allele frequencies summed for all characters (Smith, 1984). Genetically diverse parents produce high heterotic effects and yield desirable segregates. The pattern of genetic relationships between accessions can be shown by multivariate analyses. Cluster analysis is a useful statistical tool for studying the relationships among closely related accessions.

The quality of grain sorghum is determined by nutritional quality and anti-nutritional factors, such as tannins, processing characteristics, cooking quality and consumer acceptability (Hulse et al., 1980). Hence, it is important to assess genetic diversity based on quantitative and qualitative traits and identify promising accessions for different traits that could be utilised in breeding programmes.

Thus the objective of the study was to ascertain the extent of the genetic diversity underlying their biochemical and physiological characteristics that included starch (%), amylose (%), amylopectin (%), proteins (%), tannins (mg/100 ml), yield (Kg/ha) and height (cm). These traits are important because they have a direct effect on the use of sorghum varieties for different industries like baking, brewing, animal and poultry feed and other industries.

MATERIALS AND METHODS

Thirty sorghum varieties collected from the East African region were provided by ICRISAT Kenya (Table 1). The mid lowland sorghum was grown in Kampi Ya Moto (035° 56' E and 00° 05' S) at an altitude of 1660 m while the highland sorghum was grown at Egerton University altitude 2,250 m. The sorghum materials was grown in a randomized complete block design (RCBD) and replicated three times during the April to August season. The grain from two middle rows in each four row of the experimental unit was harvested, dried, threshed and used for subsequent laboratory and industrial tests.

Determination of protein content

Total nitrogen and protein was determined using Kjeldahl method (AOAC, 1999). Sorghum grain was finely milled and 0.1 g was transferred into a digestion tube. Selenium catalyst mixture weighing 1g was mixed with the sample and 5 ml of sulphuric acid (96%) was added into the tube. The tubes were then heated slowly in the digestion apparatus, until the digest was clear. The sample was transferred to a 100 ml volumetric flask, and distilled water was added into a 100 ml graduated flask. A boric acid indicator solution (5 ml) was then transferred to 100 ml conical flask containing 5 drops of mixed indicator and was placed under the condenser of the distillation apparatus. The clear supernatant (10 ml) was then transferred into the apparatus and 10 ml of 46% sodium hydroxide was added and then rinsed again with distilled water. Colour changed from pink to green when the first distillation drops mixed with the boric acid indicator solution. A total of 150 ml of the distillate was collected and titrated with 0.0174N sulphuric acids until the colour changed from green to pink. The titer volume was then read. Total nitrogen (N) was then determined as follows:

$$\% N = \left\{ \frac{a \times N \times M_w \times 100}{b \times c} \right\} \times 100\%$$

Where, a = ml of sulphuric acid used for titration of the sample,
N = Normality of sulphuric acid (0.0174),
a = Titer volume (10 ml),
M_w = Molecular weight of N_2 (0.014),
c = ml digest taken for distillation (10 ml),
b = g sample taken for analysis (0.1 g),
% crude protein = 6.25 × % N.

Determination of starch content

Percent starch content was estimated by the Anthrone method (Hodge and Hofreiter, 1962). A powdered sample (0.25 g) was homogenized in hot 80% ethanol to remove sugars. The residue was then centrifuged and dried well over a water bath. To the residue, 5.0 ml of distilled water and 6.5 ml of 52% perchloric acid was added, and then extracted at 0°C for 20 min. The supernatants were centrifuged, pooled and made up to 100 ml. Of this supernatant, 0.1 ml was pipetted out and made up to 1 ml with distilled water. The standards were prepared by taking 0.2, 0.4, 0.6, 0.8 and 1 ml of the working solution and the volume made up to 1 ml in each tube with water. To these standards, 4 ml of anthrone reagent was added to each tube and the sample heated for eight minutes in a boiling water bath. The sample was cooled rapidly and the intensity of green to dark green colour was read using a spectrophotometer at 630 nm. The glucose content in the sample was determined using the standard calibration graph, and then the value was multiplied by a factor of 0.9 to determine starch content.

Table 1. A list of the 30 selected sorghum varieties with high and low tannin, starch, protein, yield and height amounts used in this study.

Code	Variety	Code	Variety	Code	Variety
1	Ainamoi #1	11	IS 25547	21	ICSA 276 X ICSR 162
2	Siaya # 24-2	12	SDSH 90003	22	1S 25546
3	Kipkelion # 2	13	UasinGishu #1	23	IESH 22012
4	Nyiragikori	14	1S 25561	24	ICSA 12 X WAHI
5	Kipkelion # 1	15	UasinGishu #2	25	SDSA 29 X KARI MTAMA 1
6	MB 27	16	IESH 22006	26	IESV 92033 SH
7	Kabamba	17	Gadam Hamam	27	IESV 91104 DL
8	Nyangezi	18	ICSA 276 X ICSR 38	28	ICSA 371 X ICSR 108
9	BM 32	19	Kisanana	29	Busia # 21
10	1S 11162	20	Siaya # 2-3	30	IESV 92043 DL

Determination of Amylose content

Amylose was determined using the Mc Cready, (1950) method where a sample (0.1 g) of the powdered flour was weighed, and 1 ml of distilled ethanol added followed by 10 ml of 1 N NaOH. The sample was heated for 10 min in a boiling water bath. The volume was made up to 100 ml. To a 2.5 ml extract, 20 ml of distilled water was added followed by three drops of 0.1% phenolphthalein. Dropwise HCl 0.1N was then added until the pink colour disappeared. To this solution, 1 ml iodine reagent was added till the volume was 50 ml and the colour read at 590 nm using a spectrophotometer. Standard amylose working solution 0.2, 0.4, 0.6, 0.8 and 1 ml was taken and the colour developed as in the case of the test samples. The amount of amylose present in the sample was calculated using the drawn standard graph. The blank was obtained by diluting 1 ml of iodine reagent to 50 ml with distilled water. Amylose content was obtained thus:

$$\% \text{ amylose } = \left[\frac{x}{2.5}\right] \times 100 \text{ mg amylose}$$

Where *x* is the absorbance obtained.
The amylopectin content was obtained thus: % Starch - % Amylose.

Determination of tannin content

A sample of 0.5 g of the milled flour was weighed and transferred to a 250 ml conical flask, and then 75 ml of water added. The flask was heated gently and boiled for 30 min, then centrifuged at 2000 rpm for 20 min. The supernatant was collected in a 100 ml volumetric flask. A measure of 1 ml of the sample extract was transferred to a 100 ml volumetric flask containing 75 ml water. Five ml of folin reagent and 10 ml of 35% sodium carbonate solution were added, and then diluted to 100 ml with water. The sample was shaken and the absorbance read at 700 nm after 30 min. A graph was prepared using 0 - 100 mg tannic acid, where 1 ml contained 100 mg tannic acid. The tannin content of the sample was calculated as tannic acid equivalent from the standard curve. Tannins content was determined by the Folin-Denis method (Schanderl, 1970).

Determination of height and yielding ability

The height (cm) of the mature sorghum varieties was determined by measuring the average of three plants in each plot of each variety in every treatment, then the average of each plot in the three treatments.

The yielding ability (Kg/Ha) of sorghum varieties was determined in grams by getting the average yielding ability of three plants in each plot of each variety in every treatment, then the average yield of each plot in the three treatments.

Data analysis

Multivariate analysis was undertaken using JMP statistical software, version 10. Principal component analysis (PCA) was used to correlate the standardized data from biochemical composition analysis with genetic background and physiological characteristics. The determination of genetic dissimilarity was done using Euclidean distance and the hierarchial agglomerative clustering methods (Shergo et al., 2013). Euclidean measure of distance was used for the estimation of Genetic Distance (GD) among varieties.

RESULTS AND DISCUSSIONS

Principal component analysis

The biochemical composition of 30 genetically diverse sorghum varieties was measured in order to identify varieties for breeding programs. Components with an eigenvalue of less than 1 were eliminated because they were not significant (Chatfield and Collins, 1980), while those with eigenvalue greater than one, and component loadings greater than ±0.3 were regarded meaningful and significant, as reported by Hair et al. (1998). Therefore, from this study, only the first two eigenvectors, which had eigenvalues greater than 1, and cumulatively explained 69.6% of the total variation among the seven biochemical compositions describing the varieties, were considered (Table 2). Thus the principal component 1 (PC1) had an eigenvalue of 3.39 and accounted for 48.4% of the variation. This represents an equivalent of 6 variables and showed that starch, amylopectin, protein, tannins, yield, and height significantly contributed to the variation among varieties. The ones with high PC1 scores therefore contain the high levels of this biochemical

Table 2. Principal component analysis of starch, amylose, amylopectin, proteins, tannins, yield and height in sorghum varieties showing eigenvectors, eigenvalue and their percentage contribution to the total variation in the first two principal component axes.

PC	Total variance			Eigen vectors (loading) for						
	Eigenvalue	Individual %	Cumulative %	Starch	Amylose	Amylopectin	Protein	Tannins	Yield	Height
1	3.39	48.45	48.45	0.854	-0.28	0.89	-0.78	0.60	0.45	0.76
2	1.48	21.21	69.66	0.34	0.58	0.21	-0.01	0.52	-0.72	-0.41

parameters. PC2 had an eigenvalue of 1.48 contributing 21.2% of the variation that represents five variables (starch, amylose, tannins, yield and height), which contributed to the variation among the varieties. Shergo et al. (2013) found a genetic variation in 30 sorghum land races from Ethiopia using multivariate analysis. They discovered that multivariate analysis was useful measure of genetic variability among landrace accessions to single out potential donors or parental lines for future sorghum quality improvement.

The biplot (Figure 2) classifies the varieties with biochemical characteristics explained by the first two dimensions PC1 and PC2. As a result a breeder can easily pinpoint distances between the varieties and make decisions based on the principal component simultaneously. In the score plots, varieties close to each other are similar while the ones found near the origin are distinct and the ones further out are extremes. PCA analysis classifies the varieties into groups over the four quadrants based on the concentrations of these seven parameters. The varieties were distributed throughout the quadrant demonstrating large genetic variability in their biochemical contents. The ones on the top left quadrant were related in their amylose contents while, the right top quadrant contained varieties related in their tannin, starch and amylopectin contents. The left bottom quadrant and the right bottom contain varieties related in their protein, yield and height, respectively. The distance between the locations of any two varieties on the score plot is directly proportional to the degree of similarity/difference between them as per their biochemical components (Shergo et al., 2013). The score plot showed that varieties *Kisanana (#U), Ainamoi #1 (#D), IS11162 (#A), IS25546 (#B), IESV 92043 DL (#P), ICSA 371 X ICSR 108 (#K)* were the most divergent from the major group, which in the PCA as concentrated on the center demonstrating some similarity in their biochemical values. Varieties which overlapped in the principal component axes had similar biochemical contents. Thus *Kisanana (#U)* and *IS25546 (#B)* showed similar relationship in the first principal component axis.

The loading plot (Figure 1) shows the correlation among biochemical compositions. The elements with small loading located near the origin have little influence on data structure while those with high loadings represent the greatest influence on data structure on clustering and separation of sorghum varieties. Thus, all the parameters in this study had significant influence of the data structure with starch, amylopectin and tannins having influence on most varieties.

Genetic distance and cluster analysis

The genetic distance estimates matrix based on the biochemical characteristics for all pair wise combinations of (30×29)/2 = 435 of the 30 sorghum varieties is shown in Table 3. Genetic distances from 0.74 - 6.42 was observed in the pair wise combinations demonstrating that the varieties were diverse in the measured traits as their biochemical composition. The minimum genetic distances of 0.74 and 0.91 was observed between varieties *IESV 92043 DL* and *IESV 91104 DL* and between *ICSA 276 X ICSR 162* and *IESH 22006*, respectively. The highest recorded genetic distance 6.42 was between *ICSA 371 X ICSR 108* (Hybrid) and *Kabamba* (OPV) varieties, 6.30 between *SDSA 29 X KARI MTAMA 1*(Hybrid) and *Kabamba* (OPV) and 6.00 between *ICSA 371 X ICSR 108* (Hybrid) and *Ainamoi #1* (OPV) demonstrating their high genetic diversity between the varieties as a result of their biochemical contents. The wide genetic distance in this data set gives sufficient evidence of the presence of genetic diversity for the determined biochemical characteristics with little relatedness. Therefore varieties that possess with the highest genetic distance between them can be used in breeding programs crossing blocks.

Cluster analysis of biochemical composition (Figure 3) revealed a clear separation between the varieties. It showed the differences between clusters by summarizing cluster means for the seven biochemical traits. The highest cluster mean (Table 4) were recorded in yield (4153.25 Kg/ha) while the lowest were in proteins (8.9%) and amylose (17.7%). The existence of maximum genetic divergence among varieties was observed in clusters 2 and 3 due to their high cluster means. The varieties in this study were divided into four main clusters in the dendrogram. The first cluster contained varieties with the lowest starch, tannins and height as compared to the other clusters. Most varieties had a lower amylopectin to amylose ratios as compared to the other clusters. This cluster registered highest proteins and were mostly the hybrids while the yields of the varieties varied. It is useful when identifying the varieties that are favorable for

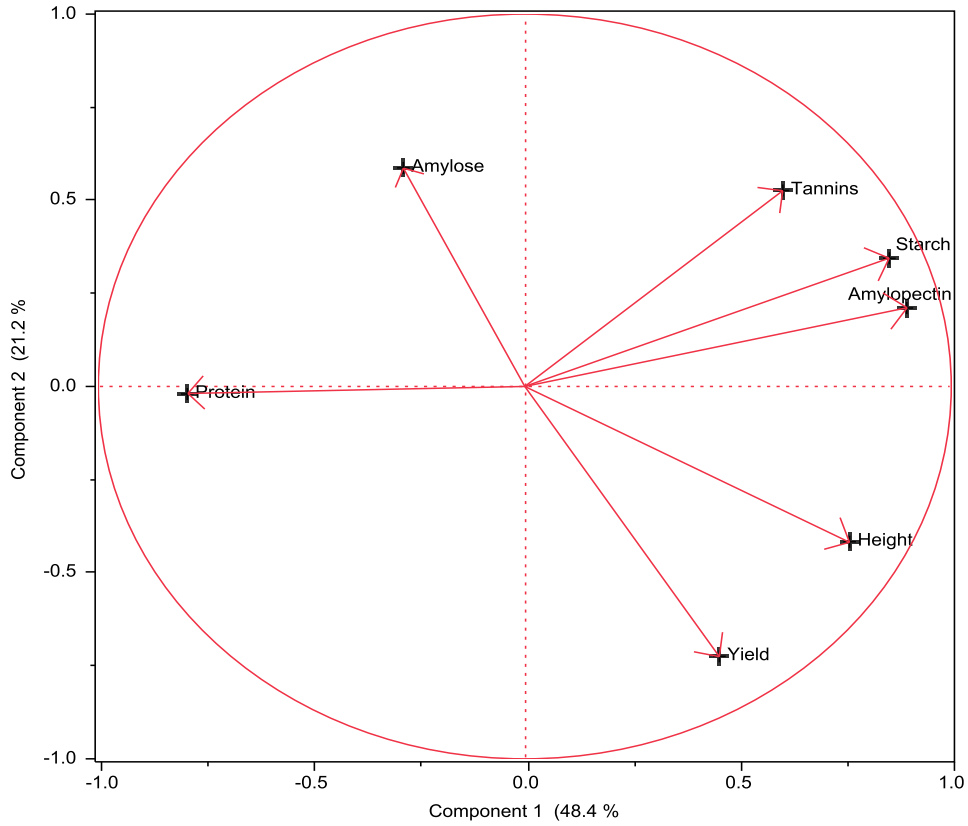

Figure 1. Principal Component Analysis loading plot for seven biochemical traits of 30 the sorghum varieties.

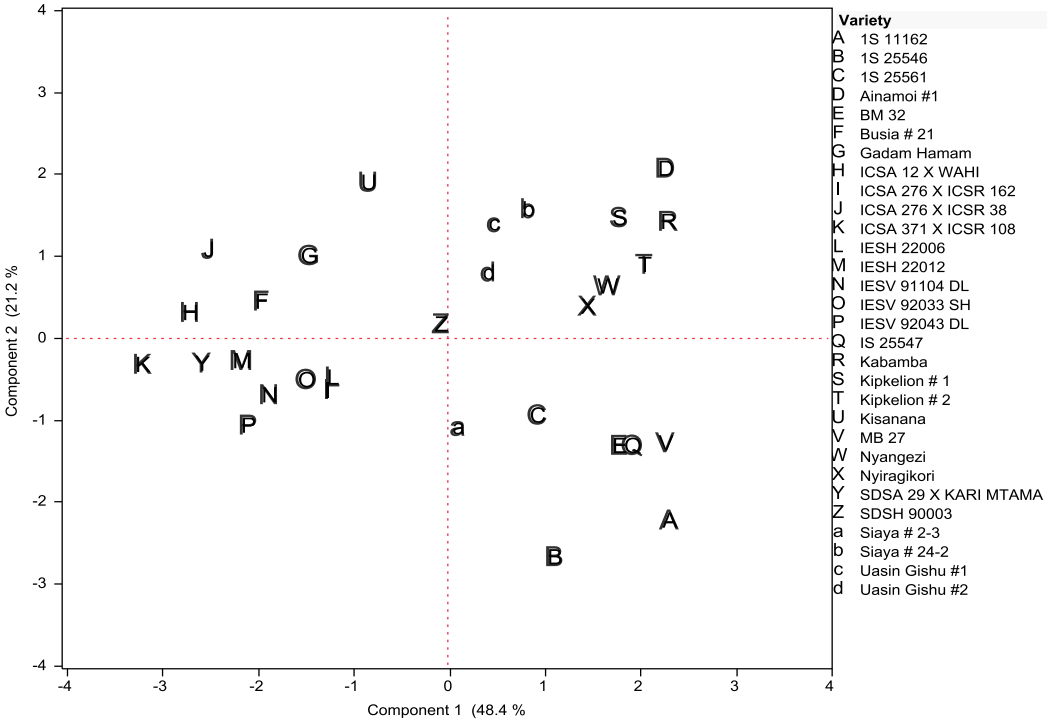

Figure 2. Principal component score plot of PC1 and PC2 describing the overall variation among sorghum varieties estimated using biochemical data.

Table 3. Estimates of genetic distance based on biochemical characteristics of 30 selected sorghum varieties from East Africa.

Genotype	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
Ainamoi #1	1																													
Siaya # 24-2	2.39	0.00																												
Kipkelion # 2	1.98	1.75	0.00																											
Nyiragikori	2.68	2.22	1.89	0.00																										
Kipkelion # 1	1.62	2.13	1.38	1.93	0.00																									
MB 27	4.05	3.42	2.67	2.84	3.31	0.00																								
Kabamba	3.21	3.53	2.27	3.72	2.56	4.27	0.00																							
Nyangezi	2.12	2.83	1.70	2.23	1.54	3.45	2.31	0.00																						
BM 32	3.45	3.64	2.96	2.72	3.26	2.38	4.53	2.69	0.00																					
1S 11162	4.33	4.44	3.38	3.36	3.97	2.48	4.50	3.11	1.49	0.00																				
IS 25547	3.52	3.81	2.79	2.78	2.95	2.24	3.94	2.29	**1.11**	1.45	0.00																			
SDSH 90003	3.53	2.02	2.66	2.71	2.81	2.84	4.24	3.14	3.02	3.90	3.10	0.00																		
UasinGishu #1	2.61	2.58	2.72	3.17	1.99	3.83	3.63	2.53	3.48	4.50	3.25	2.16	0.00																	
1S 25561	3.51	3.50	2.72	2.64	3.01	3.23	3.60	1.82	1.91	2.05	1.71	3.02	3.33	0.00																
UasinGishu #2	2.75	2.71	2.41	3.09	2.14	3.77	2.90	1.76	3.14	3.83	2.79	2.31	1.44	2.32	0.00															
IESH 22006	4.47	3.00	3.70	3.36	3.94	3.85	5.10	3.75	3.37	4.11	3.65	1.67	3.26	2.95	2.93	0.00														
GadamHamam	4.54	2.94	3.64	3.63	3.54	4.66	4.23	3.72	4.82	5.37	4.61	2.41	3.05	3.82	2.79	2.48	0.00													
ICSA 276 X ICSR 38	4.96	4.01	5.10	4.54	4.76	5.91	6.30	4.78	4.95	6.03	5.27	3.32	3.69	4.49	3.80	2.54	3.23	0.00												
Kisanana	3.90	3.98	4.55	4.66	3.76	5.52	5.35	4.06	4.65	5.86	4.64	3.36	2.00	4.52	2.78	3.81	3.86	2.97	0.00											
Siaya # 2-3	4.52	3.61	3.25	3.16	3.28	2.74	4.07	3.13	3.13	3.38	2.56	2.19	2.97	2.63	2.62	2.70	2.84	4.52	4.32	0.00										
ICSA 276 X ICSR 162	4.53	3.33	3.81	3.27	3.80	3.89	5.07	3.57	3.30	4.04	3.40	1.85	3.07	2.75	2.77	**0.91**	2.38	2.52	3.58	2.25	0.00									
1S 25546	5.08	4.96	4.24	4.28	4.53	3.05	5.14	3.76	2.11	1.96	1.84	3.66	4.22	2.50	3.63	3.73	5.13	5.58	5.21	2.80	3.49	0.00								
IESH 22012	5.32	3.78	4.42	4.12	4.64	5.02	5.22	4.27	4.65	5.07	4.70	2.95	4.15	3.53	3.49	1.76	2.01	2.90	4.60	3.29	1.87	4.75	0.00							
ICSA 12 X WAHI	5.55	4.16	4.90	4.45	4.91	5.79	5.61	4.60	5.25	5.77	5.30	3.55	4.40	4.06	3.84	2.43	2.18	2.57	4.56	3.94	2.38	5.49	**1.04**	0.00						
SDSA 29 X KARI MTAMA 1	5.44	4.40	5.17	4.53	5.09	5.64	**6.30**	4.71	4.55	5.35	4.87	3.42	4.25	3.86	3.90	2.00	3.31	1.61	3.95	4.10	1.92	4.84	2.07	1.94	0.00					
IESV 92033 SH	4.85	3.70	4.09	3.66	3.88	4.11	5.07	3.80	3.82	4.51	3.66	2.07	2.93	3.22	2.78	1.74	2.13	2.84	3.42	1.92	**1.02**	3.72	2.23	2.61	2.50	0.00				
IESV 91104 DL	5.54	4.23	4.46	4.19	4.42	4.67	4.96	4.16	4.64	4.95	4.32	2.96	3.83	3.54	3.29	2.46	1.89	3.70	4.50	2.20	1.98	4.29	1.84	2.25	3.06	1.48	0.00			
ICSA 371 X ICSR 108	**6.00**	4.88	5.59	5.08	5.39	5.99	**6.42**	5.08	5.17	5.88	5.25	3.66	4.32	4.31	4.00	2.45	3.02	2.05	3.94	3.94	2.12	5.06	2.06	1.83	1.29	2.15	2.45	0.00		
Busia # 21	5.19	4.43	4.73	5.10	4.53	5.51	4.85	4.18	4.98	5.55	4.70	3.33	3.19	3.95	2.60	3.07	2.64	3.40	3.18	3.37	2.84	4.60	2.85	3.05	3.29	2.47	2.52	2.66	0.00	
IESV 92043 DL	5.93	4.69	4.86	4.73	4.86	4.89	5.26	4.50	4.82	5.07	4.47	3.25	4.07	3.75	3.48	2.70	2.41	3.96	4.62	2.36	2.24	4.15	2.16	2.65	3.23	1.70	**0.74**	2.49	2.31	0.00

different end uses such as for the brewing and baking industry. Cluster 2 contained varieties with medium starch, height, average protein contents and high amylopectin compared to their respective amylose amounts which are lower than cluster 4 and 3. They possessed the highest yields and height compared to the rest of the clusters. Variety *MB 27* is separated from this cluster due to its low protein and tannin contents

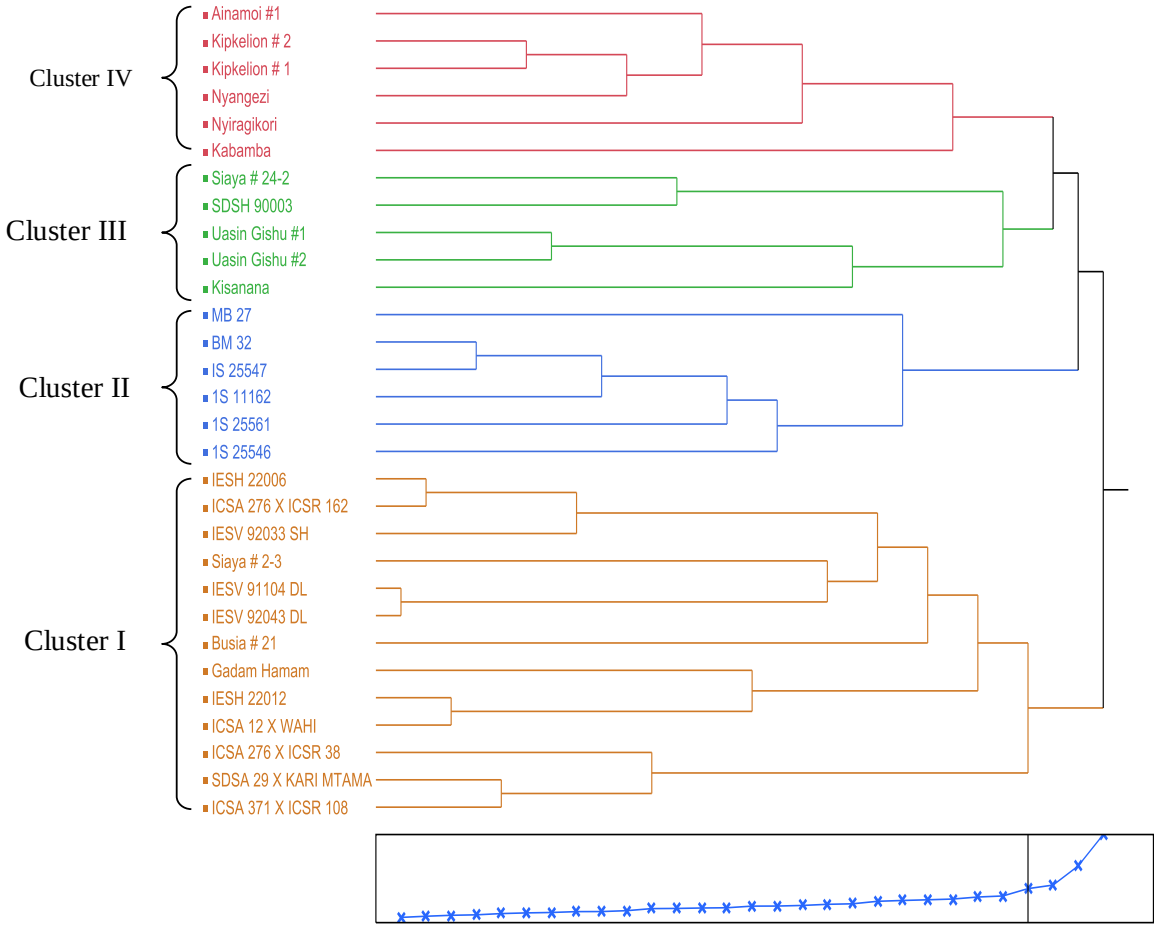

Figure 3. Dendrogram showing clusters 1 - 4 of 30 biochemical and morphological characteristics of sorghum varieties colored with red, green, blue and orange respectively.

Table 4. Summary of cluster means of 30 sorghum varieties biochemical characteristics.

Parameter	Cluster means				
	I	II	III	IV	Mean
Starch %	73.4	50.4	57.9	37.8	54.8
Amylose %	17.8	19.8	15.2	18.1	17.7
Amylopectin %	55.6	30.6	42.7	19.7	37.1
Proteins %	7.7	6.8	7.5	13.6	8.9
Tannins Mg/100 ml	61	50.3	35.1	14.9	40.3
Yield Kg/Ha	2877.2	3750.2	7058	2927.6	4153.2
Height cm	226.6	172.4	298.5	142.7	210.05
Mean	474.2	582.9	1073.6	453.5	646.0

unlike others varieties in this cluster. Cluster 3 varieties had medium starch, height and good protein amounts. They exhibited high amylopectin compared to their respective amylose, high yields and tannins. Cluster 4 varieties had the highest starch and tannins content and also had high amylopectin compared to their respective amylose amounts. They also had medium height, yield and good protein amounts. In the dendrogram *Kabamba* variety in this cluster was separated because it had the highest tannin compared to the rest. Varieties in clusters 2,

3 and 4 were predominantly open pollinated varieties with the exception of *SDSH 90003* in cluster 3.

The dendrogram showed that the varieties in this study could be valuable sources of genetic variability in sorghum breeding programmes. Multivariate analysis can therefore be a good tool for classifying biochemical characteristics. This is because, higher heterotic groups in sorghum breeding programmes in East Africa would be attained by selection and crossing of sorghum varieties in these different clusters. Grouping of these varieties according to their similarities and differences via multivariate analysis allows the breeder to better understand the data. Aremu et al. (2007) and Shergo et al. (2013) reported considerable genetic divergence among sorghum varieties on the basis of their chemical contents and phenotypic characteristics. Similar to the approach taken in the present study, Shergo (2010) also showed the grouping of accessions by multivariate method of analysis based on their divergences and similarity among sorghum varieties. The results of the present study could similarly be valuable for sorghum breeders because the most vital accessions in different clusters may be crossed with other groups to enhance the trait of interest.

Conclusion

Based on the observed variation of both quantitative and qualitative traits, it was concluded that characterizing the phenotypic diversity of sorghum varieties is important to categorize the genetic potential of varieties and could be used to increase the efficiency of sorghum breeding programmes. High genetic distances were observed among some varieties, especially between the hybrids and open pollinated varieties. The multivariate analysis successfully estimated genetic diversity among the tested varieties based on their biochemical characteristics.

Conflict of interests

The authors did not declare any conflict of interest.

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