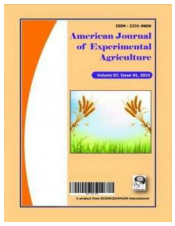

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Phenotypic Diversity of Selected Dual Purpose Forage and Grain Sorghum Genotypes

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Authors' contributions

This work was carried out in collaboration between all authors. Author SC designed the study, carried out the experiment and wrote the first draft of the manuscript. Authors TO, FK and PR reviewed the experimental design and all drafts of the manuscript. Authors SC and TO performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To study the phenotypic diversity of 25 forage and 45 grain sorghum genotypes for dual purpose as food and feed and to identify traits that might contribute to genetic improvement.
Study Design: A 7 × 10 alpha lattice design was used with two replications at two sites.
Place and Duration of Study: The study was conducted at Makerere University Agricultural Research Institute Kabanyolo (MUARIK) and National Semi Arid Resources Research Institute (NaSARRI) in Uganda between September to December, 2013 (Season 1) and April to July 2014 (season 2).
Methodology: Morphological and agronomic data were taken for each genotype from each environment in the two seasons and subjected to combined analysis of variance separately for the grain and forage sorghums. Multivariate analysis was done based on principle component and cluster analyses in which grain and forage sorghum genotypes were combined.
Results: Analysis of variance revealed significant differences *($P < 0.001$)* among the genotypes for biomass, grain yield, plant height and days to flowering indicating the possibilities of improving

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these characters through phenotypic selection. Cluster analysis grouped the genotypes into 3 clusters with cluster 1 retaining majority of the forage genotypes characterised with high biomass, Cluster 2 containing a mixture of the forage and grain sorghums characterised with high grain yield while cluster 3 contained only the grain sorghums. The first four principle components explained 89% of the total variations observed in the genotypes.

Conclusion: Based on the performance of genotypes in this study, simultaneous selection of genotypes exhibiting moderate to high levels of grain and fodder traits resulted in twelve genotypes being selected as parents for the development of dual purpose sorghum cultivars.

Keywords: Cluster; biomass; food-feed crops; principle component; variability.

1. INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is well adapted to hot and dry climates which are prone to drought and flooding because it exhibits C4 photosynthesis [1]. It is an important source of food and feed within the mixed crop-livestock production systems where its dual usage is a preferred option, especially among the resource poor small-scale farmers. Since both grain and stover are highly valued products, sorghum requires whole plant improvement rather than focusing on improvement of grain or stover traits individually [2]. The vast array of untapped genetic potential within the genus offers high possibilities of obtaining appropriate parental lines for its genetic improvement [3].

Previous attempts to improve fodder quality traits in maize have been at the expense of grain traits and vice versa [4], however, [2] demonstrated that it was possible to select for high stem biomass without compromising the improvement of grain yields in sorghum. This would suggest that positive correlations between traits linked to grain and stover yields exist. It can, therefore, be hypothesized that sorghum genotypes have high phenotypic diversity which can allow for selection of genotypes exhibiting both high grain and fodder yields for use in development of dual purpose cultivars.

Getting precise information on phenotypic diversity depends upon various estimation techniques such as plant characterization based on agro-morphological traits and using multivariate analysis approaches like cluster and principle component analysis to evaluate the magnitude of diversity among the genotypes. Multivariate data analysis presents a graphic display of the inherent latent factors and an interface between individual samples and variables that contribute to observed variations [5]. Genetic variation for morphological traits has been estimated using principal component analysis in cotton [6] and Barley [7]. [8] used cluster analysis to group winter wheat genotypes. [9] suggested using first principal component scores as input variables for the clustering process.

This study examined the phenotypic diversity of selected sorghum genotypes from East and Southern Africa to identify lines that might contribute to the genetic improvement of sorghum cultivars for dual purpose using multivariate analysis approaches.

2. MATERIALS AND METHODS

2.1 Planting Materials and Study Site Descriptions

Sorghum genotypes from ICRISAT-Kenya, Uganda, and Zambia comprising of 25 forage and 45 grain sorghum genotypes were assessed for phenotypic diversity in Uganda at Makerere University Agriculture Research Institute Kabanyolo (MUARIK) and National Semi Arid and Resources Research Institute (NaSARRI) (Table 1). MUARIK is located at 0°28'N; 32'37'E and is 1200 m above sea level with mean daily temperatures of 20°C. The site received 364.5 mm rainfall in season 1 and 561 mm in season 2. NaSARRI is located at 1°39'N; 33'27'E, and is 1038 meters above sea level with mean daily temperatures of 24°C and recorded 294.3 mm rainfall in season 1 and 538.9 in season 2.

2.2 Morphological Field Evaluation of Genotypes

The morphological characterization of the sorghum genotypes was conducted between September to December, 2013 (season 1) and April to July, 2014 (Season 2). A 7 × 10 alpha lattice design with two replications was used at each site in both seasons. Each genotype was planted in four 3 m rows, 0.6 m apart with an

intra row spacing of 0.3 m. A distance of 1 m was left between plots and 2 m between replications. Data were collected on days from planting to flowering, grain yield, 1000 seed weight, plant height, above ground biomass, Leaf-stem ratio and Leaf area (Leaf number × Leaf length × Leaf width × 0.75) following recommended sorghum descriptors [10]. Number of days from planting to flowering for each genotype were recorded when half the number of plants in the plots had flowered. To estimate plant height, the height of ten randomly selected plants was measured at the 50% plant flowering stage from the ground to the panicle tip. Leaf-stem ratio was obtained at the soft dough stage by stripping leaves off the stems of five randomly selected plants. Each was oven dried at 65℃ for 72 hours and weighed to compute the ratio.

Table 1. Selected sorghum genotypes from Kenya, Uganda and Zambia used to charecterise the phenotypic diversity

S/No	Code	Genotype name	Purpose	Source
1.	Z1 G	SDS 89426	Grain	Zambia-ZARI
2.	Z2 G	PRGC/E#69414	Grain	Zambia-ZARI
3.	Z3 G	ICSV 1089BF	Grain	Zambia- ZARI
4.	Z4 G	MACIA*DORADO	Grain	Zambia-ZARI
5.	Z5 G	ZSV-18	Grain	Zambia-ZARI
6.	Z6 G	ZSV-30	Grain	Zambia-ZARI
7.	Z7 G	ZSV-31	Grain	Zambia-ZARI
8.	Z8 G	SDS 4378-1-1-1	Grain	Zambia-ZARI
9.	Z9 G	SDS 1023-10-2-4-1-3-2	Grain	Zambia-ZARI
10.	Z10 G	SDS 876-3432(OT)8-2-1	Grain	Zambia-ZARI
11.	Z11 G	[SDS3845×SDS4548]F6-10-2	Grain	Zambia-ZARI
12.	Z12 G	[SDS3845×SDS4548]F6-10-3-2	Grain	Zambia-ZARI
13.	Z13 G	[SDS2690-2×M91057]8-2-1-1	Grain	Zambia-ZARI
14.	Z14 G	SDS 2690-2-3-5-1	Grain	Zambia-ZARI
15.	Z15 G	KSV-7	Grain	Zambia-ZARI
16.	Z16 G	KSV-10	Grain	Zambia-ZARI
17.	Z17 G	KSV-4	Grain	Zambia-ZARI
18.	Z18 G	SDS 4380-S7	Grain	Zambia-ZARI
19.	Z19 G	ZSV-12	Grain	Zambia-ZARI
20.	Z20 G	WP-13	Grain	Zambia-ZARI
21.	Z21 F	ZM 2489	Forage	Zambia-ZARI
22.	Z22 F	ZM 2499	Forage	Zambia-ZARI
23.	Z23 F	ZM 2511	Forage	Zambia-ZARI
24.	Z24 F	ZM 2518	Forage	Zambia-ZARI
25.	Z25 F	ZM 2536	Forage	Zambia-ZARI
26.	Z26 F	ZM 2547	Forage	Zambia-ZARI
27.	Z27 F	ZM 2560	Forage	Zambia-ZARI
28.	Z28 F	ZM 2562	Forage	Zambia-ZARI
29.	Z29 F	ZM 2578	Forage	Zambia-ZARI
30.	Z30 F	ZM 2580	Forage	Zambia-ZARI
31.	Z31 F	ZM 2584	Forage	Zambia-ZARI
32.	Z32 F	ZM 2592	Forage	Zambia-ZARI
33.	Z33 F	ZM 2602	Forage	Zambia-ZARI
34.	Z34 F	ZM 2610	Forage	Zambia-ZARI
35.	Z35 F	ZM 2625	Forage	Zambia-ZARI
36.	Z36 F	ZM 3869	Forage	Zambia-ZARI
37.	Z37 F	ZM 3935	Forage	Zambia-ZARI
38.	Z38 F	ZM 3990	Forage	Zambia-ZARI
39.	Z39 F	ZM 4668	Forage	Zambia-ZARI
40.	Z40 F	ZM 4856	Forage	Zambia-ZARI
41.	Z41 F	ZM 5750	Forage	Zambia-ZARI
42.	Z42 G	Sima	Grain	Zambia-Zamseed
43.	U1 G	Code 65 Plt 55 (85)	Grain	Uganda-NaSARRI

S/No	Code	Genotype name	Purpose	Source
44.	U2 G	Code 38 A-1 Plt 9 (53)	Grain	Uganda-NaSARRI
45.	U3 F	Code 22 A-2-1 Plt 99 (84)	Forage	Uganda-NaSARRI
46.	U4 F	Code 9 Plt 20 (44)	Forage	Uganda-NaSARRI
47.	U5 G	Code 90 Plt 21 (83)	Grain	Uganda-NaSARRI
48.	U6 G	Code 59 A-1 Plt 62 (71)	Grain	Uganda-NaSARRI
49.	U7 G	Code 30 Plt 446 (82)	Grain	Uganda-NaSARRI
50.	U8 G	Code 295 Plt 473 (98)	Grain	Uganda-NaSARRI
51.	U9 G	Code 18 Plt 6 (65)	Grain	Uganda-NaSARRI
52.	U10 G	Code 1 A-1 Plt 267 (80)	Grain	Uganda-NaSARRI
53.	K1 G	KARI Mtama 2	Grain	ICRISAT- Kenya
54.	K2 G	IESV 92038/2-SH	Grain	ICRISAT- Kenya
55.	K3 G	NTJ2	Grain	ICRISAT- Kenya
56.	K4 G	IESV 92008 DL	Grain	ICRISAT- Kenya
57.	K5 G	IESV 93042-SH	Grain	ICRISAT- Kenya
58.	K6 G	IS 2331	Grain	ICRISAT- Kenya
59.	K7 G	IESV 91-018 LT	Grain	ICRISAT- Kenya
60.	K8 G	IESV 92-008 DL	Grain	ICRISAT- Kenya
61.	K9 G	GADAM	Grain	ICRISAT-Kenya
62.	K 10 G	SEREDO	Grain	ICRISAT- Kenya
63.	K11 G	Malon	Grain	ICRISAT-Kenya
64.	K12 G	Raisano	Grain	ICRISAT-Kenya
65.	K13 F	Argensor 151 DP	Forage	ICRISAT-Kenya
66.	K14 F	Argensor 165 BIO	Forage	ICRISAT-Kenya
67.	K15 G	K 5989-29005	Grain	ICRISAT-Kenya
68.	K16 G	NK 7829-29006	Grain	ICRISAT-Kenya
69.	K17 G	NK 8416-19075	Grain	ICRISAT-Kenya
70.	K18 G	NK 8830-29007	Grain	ICRISAT-Kenya

ZARI-Zambia Agricultural Research Institute

2.3 Data Analysis

Analysis of variance (ANOVA) was done separately for forage and grain sorghum genotypes using the linear mixed model selection in the restricted maximum likelihood (REML) procedure in which genotypes were considered fixed. The analysis was fitted in the mathematical model as presented below;

$$Y_{ijк} = \mu + b_i + r_j + g_к + (b/r)_{ij} + (g/b)_{iк} + e_{ijк}$$

Where $Y_{ijк}$ = observed effects for ith blocks, jth replication and kth genotypes μ = grand means for the experiment, bi = effect of the ith blocks, rj = effect of jth replications gκ = effect of the kth genotype, (b/r)ij = effect of the ith blocks within the jth replication, (g/b)iκ =effect of the kth genotype within ith blocks, $e_{ijк}$ = lattice effective error or random error of the experiment.

Principle component and cluster analyses were done using pulled data for all genotypes. Cluster analysis was based on Wards clustering algorithm using Euclidean distances [11]. GenStat and R statistical programmes were used to analyse the data [12,13].

3. RESULTS AND DISCUSSION

3.1 Phenotypic Variability

Genetic variability can be inferred by phenotypic expression although the consequences of phenotypic variations depend largely on environmental changes and are further complicated by the fact that genotypes do not respond similarly to environmental changes [14]. In this study phenotypic variability among the forage and grain sorghum genotypes was observed for several traits and results of the mean sum of squares for genotypes, locations, seasons and their interactions are presented in Table 2.

The performance of the forage and grain sorghum genotypes were inconsistent at the two locations indicated by the significant *($P < 0.01$)* genotype by location (G×L) interaction effects for days to 50% flowering, weight of 1000 seeds and plant height. The interaction effects for biomass were significant *($P < 0.01$)* in the forage genotypes and also for leaf stem ratio among the grain sorghum genotypes. The genotype by season (G×S) interaction effects were

significantly different *(P < 0.001)* for all traits in both grain and forage sorghums except days to 50% flowering in the grain types and leaf stem ratio among the forage sorghums. The wider utilization of quantitative traits that exhibited significant G×L effects is limited possibly because significant G×E for a trait reduces the usefulness of the genotype over all locations for selecting and advancing superior genotypes to the next stage of selection [14].

The effects of genotype were significantly different *(P < 0.001)* for all traits except grain weight among the forage sorghums which suggested possibilities of genetic improvement through selection of elite parents with desirable traits that had no significant G×L effects. The effect of genotype on leaf to stem ratio for the forage sorghum was not influenced by location. Similar trends were observed for the interaction between genotype and season although neither forage nor grain sorghum showed any relationship between genotype and season. [15] reported variation among sorghum genotypes with respect to fodder traits. The effects of the locations were significant *(P < 0.05)* for biomass in the grain sorghum genotypes and for grain yield among the forage types. Season effects were significant *(P < 0.01)* for all traits except Leaf area in both forage and grain sorghums and days to 50% flowering and biomass in grain sorghums. The observed variation among the genotypes had genetic basis through phenotypic plasticity. Phenotypic plasticity could have been due to the differences in the rainfall, temperature and altitude of the experimental sites. Results of this study are consistent with earlier findings by [16] which indicated that the geographical pattern of quantitative phenotypic traits in Ethiopian and Eritrean sorghum gene bank accessions varied within and among geographical regions; this variation was attributed to different gradients of growing sites, rainfall and temperature that were found to be more important for genotype variations.

3.2 Cluster Analysis

The results of cluster analysis for grain and forage sorghum genotypes when the dendogram was cut at a distance of two are presented in Fig. 1. The genotypes were grouped into three major clusters with cluster 1 containing twelve forage genotypes from Zambia. Cluster 2 comprised 23 genotypes consisting of 6 forage and 17 grain sorghums, 6 of which were grain types from Kenya, 6 grain and 5 forage types from Zambia and 1 forage and 4 grain types from Uganda. The distribution of genotypes formed two sub clusters in which majority of the Zambian genotypes and 1 Kenyan line clustered together while the other sub cluster had a combination of several Ugandan and Kenyan lines. Cluster 3 had 35 Genotypes consisting predominantly of grain sorghum types and only six forage types.

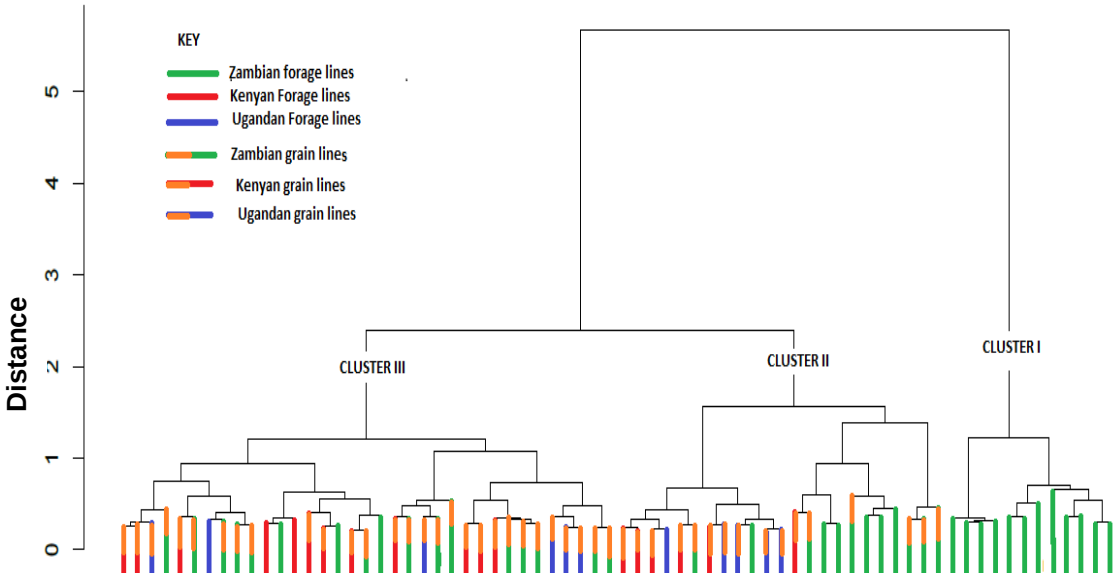

Fig. 1. Wards cluster dendogram of forage and grain sorghum genotypes

Table 2. Mean sum of squares of traits for forage and grain sorghum genotypes

Sorghum type	Source of variation	Df	Days to flowering	Plant height (m)	Leaf area (m^2)	Leaf-stem ratio	1000 seed wt (g)	Grain yld (ton ha^{-1})	Biomass (ton ha^{-1})
Forage	Genotypes (G)	24	681.6***	0.57***	0.08***	0.05***	110.0***	2.08	269.29***
	Location (L)	1	7021.1	3.93**	0.12**	0.00	8.98	12.1*	642.29
	G×L	24	159.0***	6.23**	0.63	0.01	6.5**	16.9	350.21***
	Seasons(S)	1	2132.1**	0.09***	0.01	0.00	136.9**	0.00	40.27***
	G×S	24	92.4***	0.33***	0.04***	0.00	26.8***	0.00	141.66***
	Error	96	33.35	0.03	0.01	0.02	2.9	0.94	10.84
Grain	Genotypes (G)	44	144.24***	0.41***	0.03***	0.18***	145.35***	94.31***	67.96***
	Location (L)	1	39.34	0.33	0.02	0.04	268.56**	204.17	2417**
	G×L	44	70.65***	70.65***	0.01	0.01**	12.76***	16.9	7.59
	Seasons (S)	44	1420.07	3.6***	0.03	0.00	77.41***	0.00	27.9
	G×S	44	60.9***	60.9***	0.02***	0.00	32.26***	0.00	48.59***
	Error	176	22.6	22.6	0.01	0.004	0.71	26.4	14.33

*** = significant at 0.001, ** = significant at 0.01, * = significant at 0.05

The cluster trait means of the genotypes are presented in Table 3.

Cluster one comprised forage genotypes whose flowering duration was the longest with an average of 93 days. The genotypes in this cluster had mean height of 1.81 m. The cluster had the lowest average grain yield of 2.45 tonnes ha -1 but biomass was highest at an average of 27.24 tonnes ha -1. Duration of days from planting to flowering averaged around 77 and plant height was 1.35 m for genotypes in cluster two. Grain yield was highest in this cluster at a mean of 3.76 tonnes ha -1 and biomass was 17.8 tonnes ha -1. Genotypes in cluster three had the lowest flowering duration averaging at 73 days. The maximum plant height was 1.18 m. The genotypes had the highest 1000 seed weight at a mean of 27.01 g. Average grain yield and biomass were 3.10 and 3.28 tonnes ha -1 respectively.

Highest fodder yield means were observed in the first cluster because this cluster contained predominantly forage sorghums while highest grain yields were observed in the second cluster. Grain sorghum genotypes from the three countries were very closely related implying that exchange of cultivars for breeding within these countries is unlikely to yield useful results. This low differentiation clearly was due to gene flow as grain sorghums are more frequently exchanged in these regions. Similar observations were made by [17] who conducted genetic diversity studies on grain sorghum in Kenya. However, the distinctness in clustering patterns of forage and grain sorghums indicated the existence of clear genetic divergence between the grain and forage sorghums. [18] also noted the distinct clustering pattern of different sorghum genotypes.

3.4 Principle Component Analysis (Pca)

Results of the combined principle component analysis of forage and grain sorghum genotypes are presented in Table 4. The PCA grouped the observed phenotypic traits into four groups which accounted for 89% of the total variation. [19] suggested that Eigen values greater than 1 were considered significant and component loadings of ± 0.3 were considered meaningful. Therefore in this study, the first four principle components were selected and Eigen loadings of ± 0.3 were considered as major contributory factors to the variations that were observed.

Table 3. Cluster means for traits of forage and grain sorghum genotypes

Traits	Clusters		
	1	2	3
	n = 12	n = 23	n = 35
Days to flowering	93	77	73
Plant height (m)	1.81	1.35	1.18
Leaf area (m^2)	0.51	0.45	0.37
Biomass (tonnes ha^{-1})	27.24	17.80	13.28
1000 seed weight (g)	22.55	25.62	27.01
Leaf-stem ratio	0.27	0.39	0.39
Grain yield (tonnes ha^{-1})	2.45	3.76	3.10

The first principle component accounted for 43.92% of the observed variation which was mainly due to the high positive vector loadings of days to 50% flowering, plant height, leaf area, biomass and negative loading of 1000 seed weight. [20,9] reported a large contribution of the first principle component to total variability while studying different traits. The diversity in the second principle component (16.77%) was due

Table 4. Principal component analysis (PCA)

Principle components	1	2	3	4
Eigen vectors (Loadings)				
Day to flowering	0.48	0.28	-0.17	0.03
Plant height (m)	0.49	0.23	0.05	0.18
Leaf Area (m^2)	0.35	0.50	0.11	0.38
Biomass (tonnes ha^{-1})	0.53	0.00	0.04	0.08
1000 Seed weight (g)	0.30	0.08	0.08	0.85
Leaf stem ratio	0.23	0.74	0.22	0.24
Grain yield (tonnes ha^{-1})	0.04	0.20	0.95	0.21
Eigen values	3.07	1.17	1.02	0.99
Propn of variance (%)	43.92	16.77	14.56	13.93
Total variation (%)		60.69	75.25	89.18

to positive loadings of flowering duration, leaf area and leaf-stem ratio. Grain yield was the major contributor (14.56%) to the third principle component while the 13% variation in the fourth component was due to the high negative vector loading of 1000 seed weight.

The results clearly demonstrated the amount of variation for the traits among the materials being studied which could be utilized in the selection of parental lines aimed at improving sorghum grain and forage yields for dual purpose. [20] alluded to the assumption that maximum variation yields maximum heterotic effects.

4. CONCLUSION

The study showed that phenotypic traits can classify the genotypes according to their genetic similarity or difference. Selection of genotypes based on major contributory traits for high grain and fodder simultaneously for use as parents was possible. Subsequent hybridisation of sorghum genotypes from different clusters would provide a generation of dual purpose sorghum genotypes. Their segregating progenies will likely yield good recombinants for the desired traits. Four grain (Z17, Z20, Z42 and U3) and eight forage (Z22, Z24, Z29, Z31, Z34, Z35, Z40 and Z41) sorghum genotypes were identified as ideal parents for developing dual purpose cultivars.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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