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Comparative genetic diversity and nutritional quality variation among some important Southern African sorghum accessions [*Sorghum bicolor* (L.) Moench]

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Abstract

Determination of genetic diversity and nutritional value is useful for varietal improvement. Fourteen sorghum accessions, six from Malawi (MW), four each from Tanzania (TZ) and Zambia (ZMB) considered most common and widely grown varieties in those countries were assessed for genetic diversity based on ten SSR loci and grain-Fe, Zn, total protein and starch contents. Sorghum accessions exhibited significant variation for protein, total starch content and grain-Zn ($p<0.001$) and grain-Fe ($p<0.05$). Grain-Fe content ranged from 2.8 to 6.3 mg/100g and grain-Zn content ranged 2.3 - 5.5 mg/100g. Grain protein content ranged from 9.7 to 16.3%. TZ4031 from Tanzania was superior in grain-Zn content while MW734 from Malawi was high in grain-Fe and protein contents. Zambian accessions were significantly higher in total starch content. Similarly, Tanzanian accessions were significantly superior in grain-Zn content. Protein content was significantly positive correlated with grain-Zn ($r = 0.42$). Significant and highly positive correlation ($r = 0.49$) was revealed between grain-Fe and Zn content. Nei's gene diversity revealed higher genetic variation within Malawian accessions than in other accessions used in the study. The lowest within accessions genetic diversity was exhibited by Tanzanian accessions. Cluster and principal coordinate analyses revealed similarity between Malawian and Tanzanian accessions. The results suggest presence of genetic diversity for grain-Fe, Zn, protein and starch contents for food purposes and as resource for varietal improvement. MW734, TZ4031 and TZ3966 were identified as a potential resource material for grain-Fe and Zn variety enrichment programme. However, further research is recommended for evaluation of the compositional stability of potential sorghum accessions across various environmental conditions.

Keywords: Sorghum, accessions, micronutrients, Fe, Zn, protein, total starch.
Abbreviations: AAC-Among accessions; WAc-Within accessions; AG-Among accessions; AAcWG-Among accessions within groups; ANOVA-Analysis of variance; AMOVA-Analysis of molecular variance; DNA-Deriboxynucleic Acid; Fe-Iron; h-Nei's gene diversity; He-expected heterozygosity; Ho-observed heterozygosity; I-Shannon's information index; MW-Malawi; na-number of alleles; PL-Percent polymorphism; SSR-Simple Sequence Repeats; TZ-Tanzania; TKW-Thousand kernel weight; ZMB-Zambia; Zn-Zinc.

Introduction

Sorghum [*Sorghum bicolor* (L.) Moench], a diploid with 2n = 20, is the fifth most important cereal crop worldwide after rice, wheat, maize and barley (FAO, 2011). It is a food security crop providing dietary staple for many people, especially in the semi-arid tropics (SAT). The crop's wide adaptability to conditions such as drought, water logging and salinity makes sorghum a crop of choice in marginal soils where growth of other cereals such as maize cannot be supported (ICRISAT, 1996). Mostly, local sorghum landraces demonstrate their worth for drought tolerance as they exhibited greater dry root weight, lengthy roots and higher root: shoot ratios (Ali et al., 2009). Sorghum is a principal source of energy, protein, vitamins and micronutrients for the people of semi arid tropics (Duodu *et al.*, 2003). In fact, plant proteins are the dominant and in many cases the only source of protein for people in most underdeveloped countries (Millward, 1999). Starch, the most abundant polysaccharide of plants after cellulose, is a major food reserve providing energy often at a low cost in the human diet and having diverse applications both in food and non-food industries. It is the major component (70%) of dry grain and predominantly occurs in the endosperm tissue (Benmoussa et al., 2006). In sorghum, the amylose content of starch is affected by environmental as well as genetic factors (Beta and Corke, 2001). Proteins, on average, make up 12% of dry weight of the sorghum grains. Deficiencies of micronutrients are a major global health problem and more than 2 billion people in the world are estimated to be deficient in key vitamins and minerals, particularly vitamin A, iodine, iron and zinc (FAO, 2011). Sub-Saharan Africa is reported to have the highest prevalence of nutritional related ailments in the world (FAO, 2008; Reddy Belum et al., 2005). A large

proportion of people in this part of Africa, especially the rural communities live on a diet composed primarily of staple foods prepared from cereals, tubers and plantains (Oniang'o et al., 2003). Sorghum, which is adapted to harsh growing conditions could play a significant role in the improvement of the micronutrient availability to the local people (Kayode et al., 2005). Several strategies have been suggested as intervention programmes for the reduction of micronutrient malnutrition in human populations (Maberly et al., 1994). They include food fortification as done in salt, sugar, cereals, milk and tea; dietary supplementation by use of iodized oil, vitamin A capsules and iron tablets. Other strategies are dietary diversification through consumption of red meat, liver and adequacy of vitamin C and micronutrient biofortification programmes through plant breeding. Comparatively, plant breeding has been identified as being potentially more sustainable and less expensive, since seeds could reach a larger number of people without necessarily changing consumers' behavior (Mayer et al., 2008). Screening of local sorghum germplasm with a goal of identifying suitable parents for crop breeding is the first step in the process (Bouis, 2000). There is a need to exploit the existing potential residing in the locally adapted sorghum germplasm for improved human nutritional value. The objective of this study, therefore, was to investigate the genetic diversity based on microsatellite markers and determine protein, starch, Fe and Zn contents of sorghum accessions from Malawi, Tanzania and Zambia.

Results

Fe, Zn, protein and total starch contents of sorghum grains

Grain Fe, Zn, protein and total starch contents of sorghum accessions on dry weight basis are presented in Table 1. Sorghum accessions exhibited significant variation in protein, total starch, grain Zn contents and 1000 seed weight ($p < 0.001$) and grain Fe content ($p < 0.05$). Grain Zn ranged from 2.3 to 5.5 mg/100 g whereas grain Fe ranged from 2.8 to 6.3 mg/100 g. Protein content ranged from 9.7 (TZ4255) to 16.3% (MW734).

Of the accessions used in the study, TZ4031 was superior in Zn content (5.5 mg/100 g) and the second highest in protein content (15.9%) after MW734. Total starch ranged from 53.3 (MW734) to 86.2 g/100 g (ZMB6986). Comparatively, ZMB6986 had significantly higher total starch content (86.2 g/100 g) than any other accession used in the study (Table 1). ANOVA revealed no significant differences for protein and grain Fe contents according to country of origin ($p < 0.05$; Table 2). Sorghum accessions from Zambia were significantly higher ($p < 0.05$) in total starch content than other accessions used in this study. ANOVA also revealed that sorghum accessions from Tanzania were significantly higher in grain Zn content than accessions from Malawi and Zambia.

There were no significant differences in protein, total starch, Fe and Zn contents between accessions grouped according to grain colour. Similarly, no significant variation was obtained between sorghum accessions classified as low and high thousand seed weight in protein and starch contents. However, a significant relationship was found between small and large grained accessions for grain Fe and Zn contents. Sorghum accessions with small grains comparatively exhibited higher in grain Fe and Zn contents than accessions with large grains (Table 2).

As presented in Table 3, Pearson correlations revealed a positive correlation between Fe and Zn ($r = 0.49$; $p = 0.008$). Grain protein content was positively and significantly correlated with Zn ($r = 0.42$; $p = 0{,}028$).

Characteristics of microsatellite loci across sorghum accessions

All SSR loci were polymorphic and yielded a total of 47 alleles in the 14 sorghum accessions (Table 4). The number of alleles (na_L) per locus ranged from 3 (sbAGB03, Xcup05 and Xcup50) to 8 (Xcup02), and the average number of alleles per locus was 4.7. The expected heterozygosity (gene diversity; He_L) for each locus ranged from 0.282 (Xcup05) to 0.812 (Xcup02) with a mean value of 0.622. Therefore, Xcup02 was the most informative locus with information index (I_L) of 1.767 and Xcup05 was the least informative with the index of 0.510.

Genetic diversity within and among sorghum accessions

The genetic variation in each accession as estimated by number of alleles (na_{AC}), percent polymorphic loci ($\%PL_{AC}$), observed heterozygosity (Ho_{AC}), expected heterozygosity (He_{AC}), Shannon's information index (I_{AC}) and Nei's gene diversity (h_{AC}) are presented in Table 5. A Malawian accession, MW734, had the highest number of alleles ($na_{AC} = 1.7 \pm 0.67$), followed by TZ3866 (1.6 ± 0.69; Table 5), which like MW467 and ZMB6986 also exhibited a relative high percent polymorphic loci. MW467 had the highest observed heterozygosity (0.06 ± 0.11), while the highest expected heterozygosity was estimated in MW734 (0.24 ± 0.23) and TZ3866 (0.23 ± 0.25). Gene diversity within accession as estimated by I_{AC} and h_{AC} was lowest in MW1781 and ZMB3947 ($I_{AC} = 0.0$, $h_{AC} = 0.0$) and highest in MW734 ($I_{AC} = 0.35$, $h_{AC} = 0.23$). Accessions MW409, MW467, TZ3866 and ZMB6986 exhibited moderately high gene diversity (Table 1). As presented in Table 5, estimates of percent polymorphic loci (%PL), Nei's gene diversity (h), observed heterozygosity (Ho), expected heterozygosity (He), average observed alleles (na) and Shannon information index (I) based on country of origin indicated that Malawian accessions had the highest gene diversity (%P = 80, $h = 0.41 \pm 0.29$, He = na = 2.7 ± 1.23 and $I = 0.71 \pm 0.51$; Table 5). Tanzania, on the other hand, exhibited the lowest gene diversity (%P = 70, $h = 0.25 \pm 0.26$, na= 2.2 ± 1.23 and $I = 0.42 \pm 0.44$). Analysis of molecular variance (AMOVA) revealed a highly significant genetic variation ($P < 0.001$) among accessions accounting for 15.1% of the total variation (Table 6). Furthermore, analysis of molecular data on accessions grouped based on country of origin revealed a significant genetic variation between the groups (41.9%; $P < 0.001$; Table 6). Similarly, AMOVA on accessions grouped based on altitude of collection sites revealed a significant genetic variation among groups (11.8%; $P < 0.05$). However, among accessions within groups' component of variance accounted for higher variation (72.5%) than among groups (Table 6).

Cluster and matrix plot analyses

UPGMA cluster analysis based on Rogers genetic distance matrix revealed two major clusters (clusters I and II) that were strongly (100%) bootstrap supported (Figure 2). Cluster I consisted of sorghum accessions from Zambia. The high

Table 1. Country of origin, collection site data, micronutrients (Fe and Zn), starch and protein contents and thousand seed weight (TSW) for the 14 sorghum accessions.

Accession	Country	Nearest Town	Ethnic group	LAT (S)	LON (E)	ALT	Protein (%)	Starch (g/100 g)	Fe (mg/100g)	Zn (mg/100 g)	TSW (g)
MW409	Malawi	Chikwakwa	Sena	16°21'60.0"	34°41'00.0"		11.9bcd	66.8cde	2.8c	2.6fg	24.2c
MW467	Malawi	Mulanje	Lomwe	16°01'59.9"	35°25'59.9"	700	11.9bcd	76.1abc	3.3bc	2.3h	23.1c
MW679	Malawi	Majiga	Yao	16°07'12.0"	35°07'59.9"	930	11.8bcd	76.0abc	3.9abc	2.5gh	28.4b
MW734	Malawi	Machinga	Yao	14°52'00.1"	35°02'60.0"	590	16.3a	53.3e	6.3a	3.9c	26.7b
MW1781	Malawi	Ngozi TC	Chewa	15°39'00.0"	35°39'60.0"	1382	11.2cd	60.0de	3.7abc	3.0e	24.0c
MW1798	Malawi	Chitala Res.	Chewa	3°41'60.0"	34°15'59.7"	615	14.4ab	73.6abcd	3.8abc	3.0e	15.7ef
TZ3866	Tanzania	Nachingwea	-	10°07'12.0"	38°28'12.0"	190	13.5abc	66.7cde	3.9abc	3.4d	19.0d
TZ3966	Tanzania	Serengeti	Kuria	1°42'09.0"	34°32'54.9"	1520	14.5ab	70.4bcd	5.4abc	4.5b	14.4f
TZ4031	Tanzania	Ukerewe	Kerewe	2°22'40.9"	32°26'27.9"	1130	15.9a	69.0cd	5.3abc	5.5a	11.2g
TZ4255	Tanzania	Muleba	Haya	1°58'40.9"	31°32'42.7"	1205	9.7 d	63.4cde	5.8ab	2.7f	12.0g
ZMB3947	Zambia	Lundazi	Tumbuka	12°16'60.0"	33°09'60.0"	1000	11.6bcd	75.2abc	4.5abc	2.6fg	22.5c
ZMB4859	Zambia	Ikelenge	Lunda	11°08'60.0"	24°18'00.0"	1350	10.2d	84.0ab	4.1abc	3.3d	32.9a
ZMB5395	Zambia	Mkushi	Lala	13°36'00.0"	29°22'60.0"	1250	12.2bcd	72.3abcd	4.0abc	3.5d	27.2b
ZMB6986	Zambia	Chama	Senga	11°00'58.7"	33°01'52.8"	727	11.7bcd	86.2a	4.4abc	2.8ef	16.8de
Mean							12.9**	70.9**	4.4*	3.3**	21.3**
CV[a]							20.0	12.9	24.8	26.5	30.4

*significant at $p < 0.05$, **significant at 0.001,[a]Coefficient of variation, LAT(S) = Latitude (South of equator), LON (E) = Longitude (East of Meridian), ALT = Altitude above seas level in metres.

Table 2. Comparison of mean protein, total starch, Fe and Zn contents[k] in sorghum accessions based on country of origin, grain colour and grain size.

Group	Protein (%)	Starch (g/100 g)	Fe (mg/100 g)	Zn (mg/100 g)
Country of origin				
Malawi (n = 6)	13.7 ± 3.02a	67.6 ± 9.65b	3.9 ± 1.16a	2.8 ± 0.54b
Tanzania (n = 4)	13.4 ± 2.61a	67.4 ± 4.31b	5.1 ± 1.10a	4.0 ± 1.14a
Zambia (n = 4)	11.4 ± 0.90a	79.4 ± 6.40a	4.3 ± 0.62a	3.0 ± 0.36b
Grain colour				
White (n = 7)	12.8 ± 2.13a	69.0 ± 10.70a	4.0 ± 1.07a	3.0 ± 0.54a
Brown (n = 7)	13.0 ± 3.06a	72.9 ± 7.13a	4.8 ± 1.01a	3.5 ± 1.05a
1000SW[m]				
low (n = 7)	13.2 ± 2.17a	71.8 ±7.8a	4.83 ± 0.99a	3.57 ± 1.06a
high (n = 7)	12.7 ± 2.96a	70.2 ±10.37a	3.99 ± 1.04b	2.98 ± 0.54b

[k] Means ± standard deviation; means with the same superscript are not significantly different according to Tukey's range test at the 0.05 level. [m] Thousand seed weight categorized as low (11-23 g) and high (23.1 -34 g).

similarity between accessions ZMB3947 and ZMB4859, with a genetic distance of 0.001, had a strong (100%) bootstrap support. Cluster II comprised of sorghum accessions from Tanzania in sub cluster IIa and accessions from Malawi in sub cluster IIb with a moderate (71%) bootstrap value support. TZ4031 and TZ4255 were the most similar among Tanzanian accessions with a genetic distance of 0.0225 and 100% bootstrap support. The matrix plot in principal coordinate analysis generated grouping patterns that were similar to those generated through cluster analysis (Figure 3). The first two principal axes explained 42.2% of the total variation with the first principal axis explaining 24.9% of total variation.

Association of marker alleles with grain micronutrient, protein and starch contents

Three alleles, two from marker Xcup67 and the other from SbAGB02, were fixed in the sorghum accessions used in the study. Two Xcup67 alleles, 280 bp and 298 bp, were each specific to MW467 and ZMB6986 while the fixed SbAGB02 allele of size 226 bp was specific to TZ3966 for the accessions used in this study. The SbAGB02 allele exhibited close association with higher grain Fe, Zn and protein contents than the average values of 4.37 mg/100 g, 3.26 mg/100 g and 12.6% respectively. The Xcup67 allele of fragment size 280 bp was also linked to higher grain Fe content than the average mineral content. The two fixed Xcup67 alleles were associated with grain starch content higher than the average of 70.9 g/100 g across accessions. Analysis of relationships between groups of alleles based on fragment size revealed various association patterns with grain micronutrients, protein and starch contents. For example, within the locus Xcup50, the allele of fragment size 160 bp exhibited fixed form in six sorghum accessions and extinct forms in the rest of the sorghum accessions. This allele in its extinct form was associated with high grain starch, Fe and Zn contents. Xcup50 allele of size 150 bp was extinct in all the Malawian accessions and ZMB6986 but was highly frequent or completely fixed in the other accessions. In its fixed form, on average the allele showed association with moderate to high grain starch, Fe and Zn contents. Allele of size 200 bp of locus SbKAFGK1 was fixed in two accessions ZMB3947 and ZMB4859 and nearly fixed in ZMB6986, all of which were Zambian and absent in all other sorghum accessions used in the study. The fixed allele was interesting in the manner it exhibited its association with high grain starch and low protein contents.

Discussion

Genetic diversity and clustering pattern of sorghum accessions

The amount of heterozygosity across loci, which is synonymous with allelic variation, is an indicator of the amount of genetic variability which has a bearing on the survival of a species and allows organisms to adapt to changing environments. Low average proportion of heterozygote individuals (*Ho* = 0.13) was reported in twenty-five accessions of cultivated sorghum sampled from the world germplasm collection from ICRISAT (Dje et al., 2000). In the present study a low observed heterozygosity was observed within accessions which ranged from 0 to 0.06 with an average of 0.0064. The average observed heterozygosity obtained in this study was also lower than the average Ho of 0.04 that was reported earlier involving 26

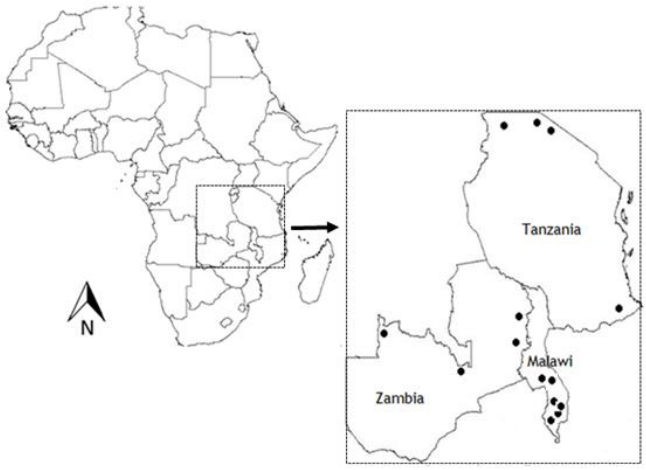

Fig 1. Map showing the localities of collection in the three countries for sorghum accessions used in this study.

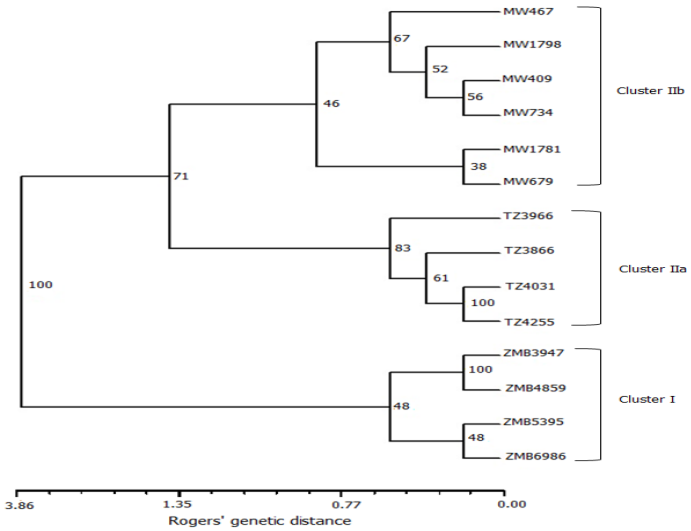

Fig 2. Genetic distance among sorghum accessions revealed by UPGMA, cluster analysis based on simple sequence repeats data with the Rogers coefficient of simgend method. The values between branches are the bootstrap values generated by 1000 resamplings in the FreeTree programme.

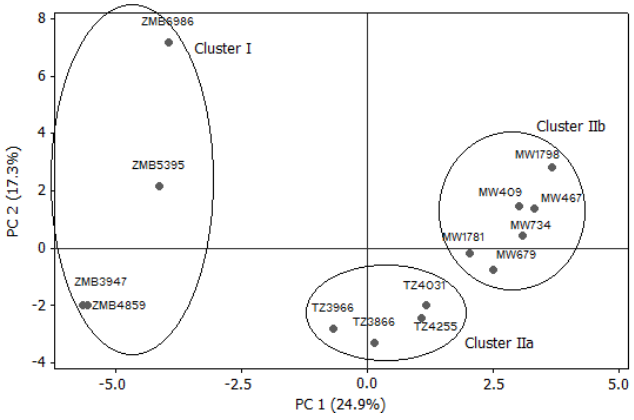

Fig 3. Principal co-ordinate analysis of 14 sorghum accessions using data for ten SSR markers. PC1 and PC2 are the first and second principal components explaining 24.9 and 17.3% of the total variation respectively.

Zambian sorghum accessions and 10 SSR loci (Ng'uni et al., 2011). The differences in number of alleles and observed heterozygosity between studies could be attributed to, among other factors, the differences in composition of germplasm material, number and type of markers and sample size used. As an example, Akter et al. (2008) identified a total of 106 alleles among 10 jute cultivars with an average of 4.61±1.92 per locus from 23 SSR primer pairs. In this study, low observed heterozygosity could largely be attributable to the predominantly inbreeding nature of sorghum relative to samples size used. In fact, when a bottleneck occurs in a population, allelic diversity is reduced faster than is heterozygosity (Nei et al., 1975), which is a result of loss of rare alleles from the population contributing little to the overall heterozygosity (Muraya et al., 2010). Sorghum is a self pollinating crop, although a wide range of out crossing rates of 7–30% or higher have been reported (Barnaud et al., 2008; Dje et al., 2004). The predominantly selfing nature of the species explains the observed lower genetic variation within than among accessions in this study as revealed by AMOVA (Table 5). Similar results have been reported from recent studies involving sorghum accession originally from Somalia (Manzelli et al., 2007) and Zambia (Ng'uni et al., 2011). Breeding systems of plant species are reported to have a significant impact on population variability with self pollinating species being the least diverse and exhibiting higher between population than within population variation (Nybom and Bartish, 2000). In fact, according to Hamrick and Godt (1996), the breeding system is one of the strongest predictors of within population genetic diversity. Low levels of genetic variation among self pollinated plant species is attributed to limited movement of genes via pollen, which also results in greater differentiation among populations (Hamrick, 1983). Based on SSR data, recent studies reported clustering of sorghum accessions according to geographic origin of germplasm (Geleta et al., 2006; Ng'uni et al., 2011). Similarly, genetic distance data from polymorphic loci in the present study clustered sorghum accessions according to their country of origin (Figure 2, 3). However, it is also evident that sorghum accessions from Zambia were genetically distant from accessions obtained from Malawi and Tanzania. The close grouping of accessions from Malawi and Tanzania suggests that the presence of relatively high levels of gene flow between sorghum populations in the two countries. Gene flow encompasses several mechanisms of gene exchange among populations, including movement of gametes, zygotes, individuals or groups of individuals from one place to another (Slatkin, 1987). In this context, seed exchange patterns between communities could be the main factor for the observed similarities among sorghum accessions of different geographical regions of origin.

Micronutrient and protein enrichment considerations in plant breeding

Dietary deficiencies of mineral nutrients are a growing nutritional problem in human populations. Iron and zinc are two micronutrients that along with pro-vitamin A (β-carotene) are recognized by the World Health Organization (WHO) as the most limiting due to their low bioavailability in diets based on cereals and legumes (WHO, 2002). Success in crop improvement through breeding depends on the existence of genetic variation for the target traits in the available gene pool. A study by Kayode et al. (2006) did not show linkage between the variation in grain-Fe and Zn content and the observed genetic variation based on AFLPs marker in Benin sorghum germplasm. Also in the present study, the patterns of association between allele frequency and grain-Fe, Zn, starch and protein contents did not show a clear linkage. However, the allelic distribution of specific SSR markers used showed some relationship with the micronutrients involved. For example, locus sbKAFGK1 belonging to sorghum linkage group, sbi5, is closely linked with protein Kafirin1 and Kafirin2 in sorghum (Kim et al., 2005). This marker, through a fixed allele of fragment size 200 bp, displayed close association with low grain protein and high starch content. This perhaps provides an indication of identification of diagnostic markers for use in breeding for improved micronutrient content. It is necessary that a relatively large sample size is involved in such studies. It is noteworthy that sorghum surpasses other major cereal grains e.g. rice, wheat, maize and finger millet, and compares well with pulse crops in terms of grain-Fe and Zn contents (Sreeramaiah et al., 2007). In preliminary studies, farmers' varieties of sorghum demonstrated potential for high grain Fe and Zn content (Jambunathan, 1980; Kayode et al., 2006). In this study, the highest Fe and Zn contents were obtained in MW734 (6.3 mg/100 g) and TZ4031 (5.5 mg/100 g), respectively. It is important to note, however, that both the environment and effects of the genotype have influence on micronutrient content of many crops as shown in wheat (Zhang et al., 2010) and grain Fe and Zn contents in sorghum (Kayode et al., 2006). Grain-Fe and Zn contents in this study were higher than 4.6 mg/100 g and 3.7 mg/100 g respectively, the levels that were reported from 29 sorghum accessions from the ICRISAT core collection (Ashok Kumar et al., 2009). The grain-Fe and Zn contents observed in the present study were also higher than the average of 5.9 mg/100 g and 2.44 mg/100 g respectively, reported from a study involving 76 farmer varieties of sorghum from Benin (Kayode et al., 2006). Grain-Fe and Zn contents higher than 5 mg/100 g and 3.7 mg/100 g respectively have been recommended for potential sorghum lines for use in the breeding programme for grain micronutrient enrichment (Ashok Kumar et al., 2009). In the present study, MW734, TZ3966 and TZ4031 had comparable or even higher than suggested grain-Fe and Zn contents and therefore have shown to have potential of being utilized in micronutrient enrichment programmes. It is necessary, though, that top ranking accessions with high grain micronutrients are evaluated in replicated trials in multiple environments to study their compositional stability and observe genotype and environment interaction for expression of these micronutrient (Velu et al., 2011). A significant and fairly high positive correlation ($r = 0.49$) was obtained for grain-Fe and Zn contents (Table 3) which has implications for the possibility to combine selection for both micronutrients in a single agronomic background. Similar relationships between Fe and Zn have been reported in sorghum (Kayode et al., 2006; Reddy Belum et al., 2005), wheat (Velu et al., 2011) and rice (Zhang et al., 2004). However, in order to realize desired impact of micronutrient-dense improved cultivars in human nutrition, micronutrients must be delivered in sorghum varieties that also meet the farmer-preferred grain traits such as early maturity, grain size and colour. The present study has indicated a negative correlation between micronutrients (Fe and Zn) and TSW, a trait for seed size. This could be attributed to the effect of dilution caused by enhanced grain starch content (Bänzinger and Long, 2000).

Table 3. Pearson correlation coefficients between protein, total starch, Fe, Zn and TSW of sorghum grains.

Trait	TSW [a]	Protein	Starch	Fe
Protein	-0.08ns			
Starch	0.13ns	-0.22ns		
Fe	-0.33ns	0.23ns	-0.34ns	
Zn	-0.38*	0.42*	-0.24ns	0.49**

*, ** indicating significant correlation at $p < 0.05$, and 0.01 respectively, [a]TSW = Thousand seed weight.

Materials and methods

Plant materials

Fourteen sorghum accessions obtained from national gene banks of Malawi (6 accessions), Tanzania (4 accessions) and Zambia (4 accessions) were used in this study (Table 1). These sorghum accessions are popular local sorghum varieties that are cultivated over wide geographical area in their respective countries (Figure 1).

Measurement of thousand kernel weight (TKW)

For each sorghum accession, thousand kernel weight was determined based on the weight of 100 dry grains weighed. These measurements were made in two replicates for each accession.

Iron and zinc determination

About 50 g of grains of each accession was milled to flour using a laboratory mill (Yellow line, A10, IKA-Werke, Staufen, Germany). Following milling, samples were freeze dried to constant dry weight over a period of four days. About 0.5 g of each flour sample was digested as described by Hussain et al. (2010). The digested samples were analyzed for mineral contents at the ICP laboratory (Department of Ecology, Lund University, Sweden) using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES; Perkin-Elmer, OPTIMA 3000 DV). Atomic spectrometry standards from Perkin-Elmer, SPEX, AccuStandard and Merck were used for this analysis.

Protein determination

Sorghum grain samples were milled and freeze dried to constant weight as for mineral content analysis prior to total nitrogen analysis. Samples were weighed (2-5 g) using 5 x 9 mm tin capsules. Capsules containing samples were rolled into pellets. An aliquot was burned in an elemental analyzer (Nitrogen analyzer, NA 1500 series 2; Micromass, Carlo Erba Instruments, Rodano (Milan), Italy) at 1020°C and interfaced with an isotope ratio mass spectrometer (Optima; Micromass) leading to the release of CO_2, H_2O and N_2. Passage of the produced gasses over special absorbent columns eliminated CO_2 and H_2O. Nitrogen content was measured by passing the remaining gasses through a column that has a thermal conductivity detector at the end. Acetanilide (C_8H_9NO; C = 71.09%, H = 6.71%, N = 10.36%, O = 11.84%) was used as the standard reference material in this assay. A protein factor of 6.25, equivalent to (1/6.25) or 0.16 g nitrogen per gram of protein, was used to estimate protein content in sorghum, as recommended by Merrill and Watt (1973).

Total starch determination

Approximately 50 mg of dry flour was weighed in duplicates and placed in a glass centrifuge tube (16 x 120 mm; 17 ml capacity). Starch content was determined using the total starch analysis protocol developed by AA/AMG; Megazyme International, Wicklow, Ireland. The enzymatic (α-amylase/amyloglucosidase) digestion of starch releases glucose, which was spectrophotometrically, quantified at 510 nm.

DNA extraction and SSR analysis

Sorghum seedlings were raised in a greenhouse. Fresh leaf tissues were sampled from the seedlings for DNA extraction at two weeks of age. DNA was extracted from ten individual plants of each accession using a modified CTAB method (Bekele et al., 2007). The quality and concentration of extracted DNA were determined as described in Ng'uni et al. (2010). The final DNA concentration of each template stock was adjusted to 10 ng/µl.

PCR amplification

Ten SSR primer pairs were used for amplification. PCR amplification was carried out in a 25µl reaction mixtures containing 10ng of genomic DNA, 10 µM of each primer pair, 10X PCR buffer, 25 mM MgCl2, 10 mM dNTPs, 5 unit Taq DNA polymerase using a GeneAmp® PCR system 9700 (Applied Biosystems, Inc, USA). A touchdown PCR programme was used. This programme involved an initial denaturation step of 3 min at 94°C followed by 10 cycles of 30 sec at 94°C, 30 sec at 65°C annealing temperature reduced by 1°C every cycle, and 45 sec extension at 72°C. This was followed by 30 cycles of 30 sec at 94°C, 30 sec at 55°C and 45 sec at 72°C. Then, the extension phase of 72°C for 20 sec and holding temperature of 4°C were followed. The PCR products were separated using readymade polyacrylamide gel (ETC Electrophorase-technik, Germany) electrophoresis and visualized using silver staining method according to Amershan Pharmacia's DNA silver Staining Kit (Amersham Pharmacia Biotech, Inc, Sweden).

Statistical data analysis

Data for protein, starch, iron and zinc contents were subjected to analysis of variance (ANOVA). Tukey's range test, a procedure for comparison of a multiple means, was applied to find which means were significantly different from the other. These statistical analyses were performed using Minitab version 16. DNA fragments for a particular locus were scored as fragment sizes allelic data in comparison with a standard 50 bp DNA ladder. The allelic data were used to estimate percentage of polymorphic loci, Shannon's information index (I) , Nei's (1973) expected heterozygosity or gene diversity

Table 4. Characteristics of SSR loci used in this study and some genetic diversity parameters estimated for each locus.

SSR locus	Chr.	Repeat Motif	Primer	na_L[a]	Ho_L[b]	He_L[c]	I_L[d]	OASR[e](bp)
Xcup64	2	$(TA)_9$	F-TATTGACACGCAGGTAACGC R-GAGGACGAGTGCATGATGAG	6	0.000	0.800	1.683	200-250
Xcup74	2	$(TG)_9$	F-GTCGCCATTGTGATGAAGAG R-CAGTAGTCCAGCAAAACGGC	5	0.014	0.688	1.267	173-187
Xcup05	4	$(GA)_8$	F-GGAAGGTTTGCAAGAACAGG R-CCAGCCCAACAAGTGCTATC	3	0.029	0.282	0.510	125-135
SbKAFGK1	5	$(ACA)_9$	F-AGCATCTTACAACAACCAAT R-AGCATCTTACAACAACCAAT	4	0.014	0.741	1.364	175-200
SbAGB02	7	$(AG)_{35}$	F-CTCTGATATGTCGTTGTGCT R-ATAGAGAGGATAGCTTATAGCTCA	5	0.000	0.578	1.145	224-240
sbAGB03	2	$(AG)_{41}$	F-GTGTGTGTAGCTTCTTGGG R-ACGTAGGAGTAGTTTCTAGGATT	3	0.000	0.482	0.839	200-250
Xcup02	9	(GCA)6	F-GACGCAGTTTGCTCCTATC R-GTCCAACCAACCCACGTATC	8	0.000	0.812	1.767	196-240
Xcup49	10	(GGAT)6	F-TCCACCTCCATCATCTTTCC R-CTCCACCACCTCCATGACTC	5	0.007	0.663	1.285	190-199
Xcup50	10	(ACAGG)5	F-TGATTGATTGAGGCAGGCAC R-TTCCGGTCTCTGTCCATTTC	3	0.000	0.569	0.912	148-160
Xcup67	10	$(TA)_6$	F-GGTCAGTGCTTACACAGATTCC R-GGGGATTGCAGGTGTCATAG	5	0.000	0.600	1.184	256-300
		Mean		4.700	0.006	0.622	1.196	
		SE		1.567	0.010	0.160	0.378	

[a] = Observed number of alleles for each locus,[b] = Observed heterozygosity for each locus, [c] = Expected heterozygosity for each locus
[d] = Shannon's Information index for each locus, [e] = Observed allele size range

Table 5. Estimates of some genetic diversity parameters for sorghum accessions per country of origin.

Accession	na[a]	%PL[b]	Ho[c]	He[d]	I[e]	h[f]
MW467	1.5 ± 0.53	50	0.06 ± 0.11	0.15 ± 0.19	0.22 ± 0.27	0.14 ± 0.19
MW1798	1.3 ± 0.48	30	0.01 ± 0.03	0.06 ± 0.12	0.10 ± 0.18	0.06 ± 0.11
MW409	1.4 ± 0.52	40	0	0.18 ± 0.24	0.25 ± 0.33	0.18 ± 0.10
MW734	1.7 ± 0.67	60	0.01 ± 0.03	0.24 ± 0.23	0.35 ± 0.34	0.23 ± 0.22
MW679	1.3 ± 0.27	30	0	0.12 ± 0.20	0.17 ± 0.27	0.10 ± 0.10
MW1781	1.0	0	0	0	0	0
TZ4031	1.3 ± 0.48	30	0	0.10 ± 0.16	0	0.10 ± 0.15
TZ4255	1.4 ± 0.69	30	0	0.12 ± 0.22	0	0.11 ± 0.21
TZ3866	1.6 ± 0.69	50	0.01 ± 0.03	0.23 ± 0.25	0.33 ± 0.36	0.22 ± 0.24
TZ3966	1.1 ± 0.32	10	0	0.05 ± 0.16	0	0.05 ± 0.15
ZMB6986	1.5 ± 0.53	50	0	0.16 ± 0.18	0	0.16 ± 0.17
ZMB5395	1.2 ± 0.42	20	0	0.07 ± 0.17	0	0.07 ± 0.16
ZMB4859	1.1 ± 0.32	10	0	0.02 ± 0.06	0	0.02 ± 0.06
ZMB3947	1.0	0	0	0	0	0
Malawi (n = 6)	2.7 ± 1.23	80	0.01 ± 0.02	0.41 ± 0.29	0.71 ± 0.51	0.41 ± 0.29
Tanzania(n = 4)	2.2 ± 1.23	70	0.01 ± 0.01	0.25 ± 0.26	0.42 ± 0.44	0.25 ± 0.26
Zambia (n = 4)	2.3 ± 0.82	90	0	0.42 ± 0.18	0	0.42 ± 0.18

[a] mean observed alleles, [b] The percentage of polymorphic loci, [c] mean observed heterozygosity, [d] mean *H*e expected heterozygosity, [e] mean Shannon information index , [f] mean Nei's gene diversity over loci.

(h), observed and expected heterozygosities according to Levene (1949) as applied by POPGENE version 1.31 (Yeh and Boyle, 1997). Genetic variation within accessions and among accessions/group of accessions was estimated through analysis of molecular variance (AMOVA) using the Arlequin 3.0 software (Excoffier et al., 2005). Cluster analysis based on Unweighted Pair Group Method with Arithmetic Average (UPGMA) using sequential agglomerative hierarchical nested (SAHN) analysis approach and principal co-ordinate analysis were performed based on Nei's (1973) distance matrix using NTSYSpc software (Rohlf 1998). To estimate the robustness of obtained trees, a bootstrap analysis of 1000 replicates was conducted using FreeTree – Freeware programme (Pavlicek et al., 1999).

Conclusion

This study assessed the pattern of genetic diversity and grain-Fe, Zn, protein and starch contents of sorghum accessions from Malawi, Tanzania and Zambia. Significant genetic diversity among sorghum accessions was evident and considerable variability among accessions for grain-Fe, Zn,

Table 6. AMOVA for 14 southern Africa sorghum accessions based on SSR data: (A) without grouping the accessions, (B) by grouping the accessions based on agroecological region I and II, (C) by grouping the accessions according to altitude as (i) 100 < Low to medium < 999 m and (ii) 1000 < high < 2000 m masl.

Groups	Source of variation[Ψ]	df	Variance	Variation (%)	P-value
(A) Ungrouped	AAc	13	Va = 2.75	83.36	***
	WAc	126	Vb = 0.50	15.13	***
(B) Countries	AG	2	Va = 1.58	41.90	***
	AAcWG	11	Vb = 1.64	43.52	***
	WAc	126	Vc = 0.50	13.25	***
(C) Altitudes	AG	1	Va = 0.41	11.77	*
	AAcWG	12	Vb = 2.53	72.49	***
	WAc	126	Vc = 0.50	14.30	***

[Ψ]AAc = among accessions; WAc = within accessions; AG = among groups; AAcWG = among accessions within groups
*, *** indicating significant correlation at $p < 0.05$ and 0.001respectively.

protein and starch contents were revealed. Comparatively, Malawian sorghum accessions in this study exhibited higher gene diversity than those from Tanzania and Zambia. This could perhaps be attributed to the differences in number of accessions from each country that was involved in the study. Given that resources are limited, when prioritizing accessions for conservation, consideration should be given to sorghum accessions that exhibit high gene diversity taking into account accessions representative of different clusters and sub-clusters. Identification of sorghum germplasm for breeding for improvement of micronutrients and protein contents seems promising. Superior accessions identified in this study should further be studied for stability and heritability of the traits under different agroecological conditions. It is important to consider accessions that satisfy farmers' preferred traits such as early maturity, grain color and yield for delivery of high grain-Fe, Zinc and protein contents.

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