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Genetic variability of sorghum landraces from lower Eastern Kenya based on simple sequence repeats (SSRs) markers

Catherine W. Muui[1]*, Reuben M. Muasya[2], Duncan T. Kirubi[1], Steven M. Runo[3] and Arthur Karugu[4]

[1]School of Agriculture and Enterprise Development, Department of Agricultural Science and Technology, Kenyatta University, P.O. Box 43844-00100, Nairobi, Kenya
[2]School of Agriculture and Veterinary Sciences, Department of Dry Land Agriculture, South Eastern Kenya University, P.O. Box 170-90200, Kitui, Kenya.
[3]Department of Biotechnology and Biochemistry, Kenyatta University, P.O. Box 43844-00100, Nairobi, Kenya.
[4]International Maize and Wheat Improvement Centre CIMMYT Kenya, P.O. Box 1041-00621, Nairobi, Kenya.

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The aim of this study was to estimate the genetic variability of sorghum landraces grown in lower eastern Kenya based on simple sequence repeats (SSRs) markers. A total of 44 landraces obtained from the farmers and four improved varieties were analyzed using 20 SSR markers. All markers were polymorphic with polymorphism information content (PIC) value ranging from 0.04 to 0.81 with a mean of 0.49. Heterozygosity ranged from 0.00 to 0.04 suggesting that each detected a single genetic locus and that each of the sorghum accession used was stable. The alleles ranged between 2 and 10 and an average of 5.05 alleles per primer pair. The gene diversity ranged from 0.04 to 0.83 with a mean value of 0.53. All possible allele combinations ranged from 2 to 10, while major allele frequency ranged from 0.32 to 0.98. Genetic distances varied from 0.15 to 0.90 with two genotypes Karuge 1 and Karuge 2 obtained from Kiritiri in Mbeere having the minimum (0.15) and indication of very close relatedness. The diversity of the landraces was structured more on geographical locations and on seed colorations than agro-ecological conditions. Such intraregional genetic proximity in sorghum landraces would arise through seed exchanges among farmers. Analysis of molecular variation indicated higher variation within populations than among the groups. The genetic diversity can be exploited in hybridization programs to improve sorghum varieties grown by farmers in semi arid areas.

Key words: Genetic variability, landraces, simple sequence repeats, sorghum.

INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is one of the important staple crops for the world's poorest and food insecure people in the semi-arid regions of the world (Timu et al., 2012; Rohrbach et al., 2002; Doggett, 1988). In Kenya, sorghum is an important food crop and dietary staple in the country's arid and semi-arid lands which account for over 80% of the total land area. Sorghum is uniquely cultivated due to its tolerance to drought, water logging, saline-alkali infertile soils and high temperature (Taylor, 2003). It has for a long time been considered as

a crop of the resource-poor small-scale farmers and is grown predominately in arid and semi-arid lands (ASALs) (USAID, 2010).

All subspecies of *S. bicolor* are inter-fertile under sympatric conditions, leading to a continuum of wild-domesticate complex forms that have been documented to occur in many sorghum growing parts of Africa (Mutegi et al., 2010; Tesso et al., 2008; Dogget, 1988; Dogget and Majisu, 1968). Therefore, a wide genetic diversity is expected in the landraces of cultivated sorghum in Africa.

In lower Eastern Kenya, a wide diversity of sorghum landraces is cultivated under diverse agro-climatic conditions and practices by subsistence farmers (Muui et al., 2013; Mutegi et al., 2010). Farmers maintain sorghum landraces unique in their adaptation, grain yield, quality of harvested products, biotic stress resistance and in postharvest processing (Muui et al., 2013).

Sorghum with diverse morpho-types have been reported in many of the sorghum growing regions of Africa, often as indistinct races of *S. bicolor* that form a crop-wild-weed complex (Ejeta and Grenier, 2005; de Wet, 1978). Lower Eastern Kenya has a diverse of sorghum seed colorations, an indication of a possibility of early existence of crop-wild-weed complex of sorghum (Muui et al., 2013). Seed exchange among resource-poor small-scale farmers is a contributing factor to high variation among sorghum landraces (Tulole et al., 2009).

Farmers grow a mixture of several sorghum landraces per field (Muui et al., 2013; Barnaud et al., 2007). Over time, outcrossing occurs in sorghum though variable among different landraces (Barnaud et al., 2008). Also, selection exerted by farmers is a key parameter for determining the fate of new genetic combinations from the outcrossing events and thus in the patterns of genetic differentiation among landraces. Landraces perform well under sub-optimal conditions as they are well adapted to local stresses and possesses farmers' preferable traits (Bantilan et al., 2004; Setimela et al., 2004). It is, therefore, necessary to study the genetic relationships of these landraces and identify traits to be incorporated in the released varieties. Estimation of genetic diversity to identify groups with similar genotypes is important for conserving, evaluating and utilising genetic resources, for studying the diversity of different germplasm as possible sources of genes that can improve the performance of cultivars, and for determining the uniqueness and distinctness of the genetic constitution of genotypes (Subudhi et al., 2002).

Levels and patterns of diversity within and between cultivated and wild sorghum gene pools have been reported (Cui et al., 1995; Deu et al., 1995; Casa et al., 2005). In Kenya, studies were done to establish the extent and direction of introgression between cultivated and wild sorghum relatives (Mutegi et al., 2010). However, the extent of genetic diversity within and between landraces grown by farmers in different agro-ecological zones of lower Eastern Kenya has not been established. Our study therefore applied microsatellite markers to analyze cultivated sorghum sampled from different growing regions in lower Eastern Kenya, in order to elucidate patterns of diversity. The objective of the study was to determine the genetic relationships and thus establish the potential for landraces as sources of breeding material for future sorghum improvement.

MATERIALS AND METHODS

This research was conducted at the lower Eastern Kenya which extends between 38° 15 E and 39° 30 E as well as 1° N and 3° S. The study covered four regions of lower eastern varying in agro-climatology, namely Mbeere, Makueni, Kitui, and Mutomo, which are major sorghum growing areas in Kenya. The regions range from zone IV (semi humid to semi arid) to zone V (semi arid) (Jaetzold and Schmidt, 1983). The Mbeere and Kitui sites are classified as Lower Midland (LM) with some regions in transitional zone towards Upper Midland (UM). Makueni and Mutomo sites are classified as LM (Jaetzold et al., 2006).

Mbeere region where sorghum is grown receives an annual rainfall ranging from 800 to 1000 mm and an altitude of 840 to 1189 m above the sea level (Jaetzold et al., 2006). Makueni area receives an average annual rainfall ranging from 600 to 800 mm and an altitude of 914 to 1600 m above the sea level. Kitui receives an annual rainfall ranging from 600 to 1181 mm and an altitude of 1036 to 1115 m above the sea level, while Mutomo receives 500 to 700 mm annual rainfall and is at an altitude of 914 m above the sea level (Jaetzold et al., 2006). Collection of landraces was done in 2010 to 2011 (Muui et al., 2013) in the major sorghum growing agro ecological zones as follows; Mbeere in Kiritiri (LM_4), Ishiara (LM_4) Siakago (LM_3); Makueni in Kibwezi (LM_5), Kiboko (LM_5; LM_6), Makindu (LM_5); Kitui in Kitui central (LM_3), Kitui west (LM_3) and Mutomo in zone 1 and 2 (LM_4; LM_5) (Jaetzold et al., 2006) (Figure 1).

Forty four sorghum landraces collected from farmers in the study region and four commercially released cultivars were used to assess the genetic diversity (Table 1). Seeds for each accession were sown in trays containing soil and seedlings raised under standard glasshouse conditions at International Crops Research Institute for Semi-Arid Tropics (ICRISAT), World Agroforestry campus, Nairobi between July and September, 2012. Leaves were taken from two weeks old individual plants, from each accession.

DNA extraction was done using cetyl trimethyl ammonium bromide (CTAB) according to Mace et al. (2003). The quality of genomic DNA was assessed using agarose (0.8%) gel and quantification was done using a spectrophotometer nanodrop according to Mace et al. (2003). Polymerase chain reaction (PCR) amplifications were performed in 60 µl reaction mixture. Twenty primers of known sequence were used in amplification of the 48 samples (Billot et al., 2012). The amplification was carried out using the profile developed by Folkertsma et al. (2005). The PCR product was then loaded on 2% agarose gel and DNA fragments were visualized by illumination device with UV light. The success of amplification was indicated by the presence of one or two sharp

*Corresponding author. E-mail: catherinemuui@gmail.com

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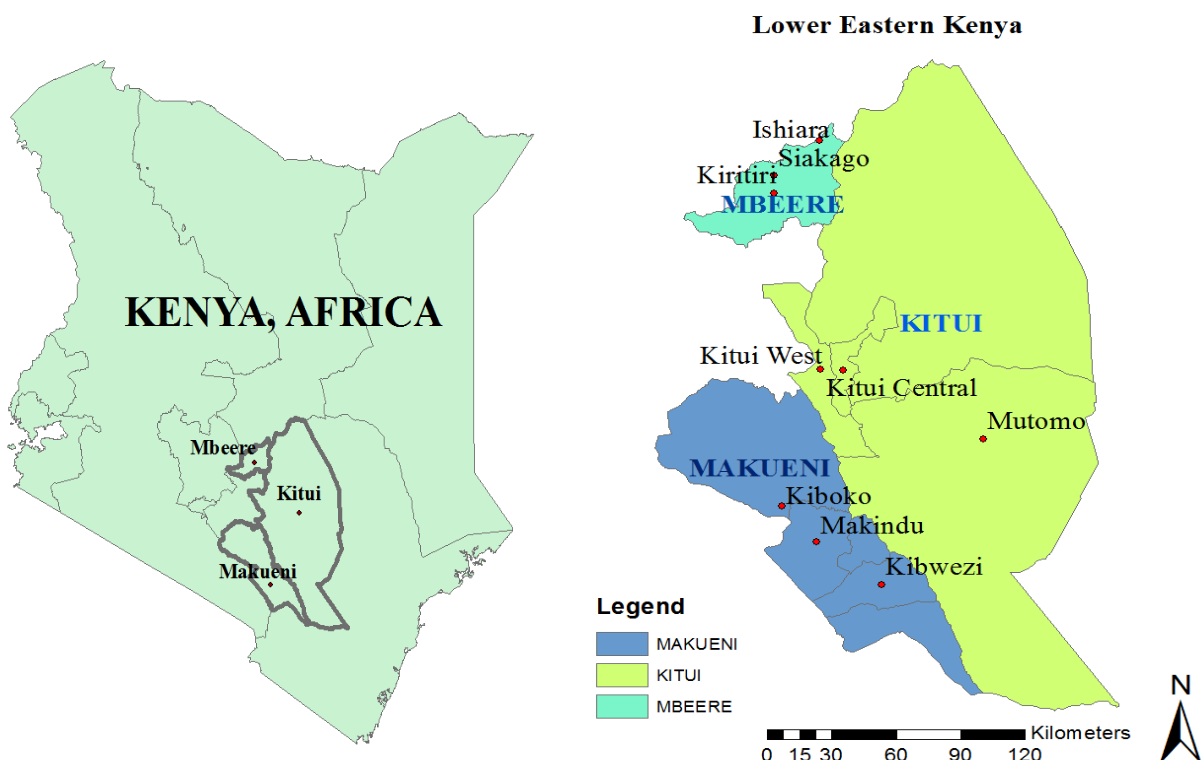

Figure 1. Map showing the study area in lower Eastern Kenya.

Table 1. Reference numbers for 44 landraces and 4 improved sorghum seed accessions used in diversity experiment.

Number	Accession identity	Number	Accession identity	Number	Accession identity
1	Kitui West white	17	Mbeere Kiritiri local A	33	Mbeere Kiritiri mubaku
2	Mbeere Kiritiri Gatengu	18	Kitui West white brown	34	Kitui Central brown white 2
3	Serena commercial	19	Mutomo black red	35	Mbeere Ishiara red
4	Mbeere Siakago white	20	Makueni Kiboko dirty white	36	Makueni Kibwezi brownwhite
5	Kitui Central brown	21	Makueni Makindu brown	37	Kitui West brown 1
6	Mbeere Kiritiri purple	22	Kitui Central white	38	Kitui West brown 2
7	Mbeere Kiritiri mwitia	23	Mutomo brown white	39	Mbeere Kiritiri thiriku 2
8	Mbeere Kiritiri ciakiondo	24	Kitui Central brown white 1	40	Mbeere Siakago red Seredo
9	Makueni Makindu white	25	Makueni Kiboko brown white	41	Gaddam commercial
10	Makueni Kibwezi brown	26	Mbeere Kiritiri thiriku 1	42	Kitui West brown 3
11	Mbeere Kiritiri karuge 2	27	Mbeere Siakago thiriku	43	Mutomo white
12	KARI Mtama 1commercial	28	Mutomo brown	44	Kitui Central brown red
13	Mbeere Kiritiri karuge 1	29	Seredo commercial	45	Kitui West white mweruba
14	Mbeere Kiritiri local B	30	Makueni Kiboko red	46	Makueni Kibwezi red
15	Makueni Makindu red	31	Makueni Kiboko brown	47	Mbeere Kiritiri muthiriku
16	Kitui Central red 1	32	Mbeere Siakago Serena	48	Kitui Central red 2

bands within the size range of up to 100 bp. Simple sequence repeats (SSR) was done by loading the PCR products for DNA fragments denaturation and size fractioning using capillary electrophoresis. Fragment size fractioning was done using ABI 3730 automatic DNA sequencer (Perkin Elmer-Applied Biosystems). Genemapper software Version 4.0 (Perkin Elmer-Applied Biosystems) was applied to size peak patterns, using the internal ROX 400 HD size standard and for allele calling.

Statistical analysis

Using the binned dataset, PowerMarker v.3.25 (Liu and Muse, 2005) was used to calculate the total numbers of alleles, the numbers

Table 2. Characteristics of 20 SSR markers across 44 sorghum landraces and 4 commercial varieties.

Marker	Repeat motif	Major allele frequency	Genotype number	Allele number	Availability	Gene diversity	Heterozygosity	PIC
gpsb067	(GT)10	0.55	4	4	0.98	0.57	0.00	0.50
mSbCIR238	(AC)26	0.54	4	4	0.96	0.61	0.00	0.54
mSbCIR248	(GT)7.5	0.36	4	4	0.98	0.72	0.00	0.67
mSbCIR276	(AC)9	0.83	5	5	0.98	0.30	0.00	0.29
mSbCIR283	(CT)8 (GT)8.5	0.58	4	4	1.00	0.58	0.00	0.52
mSbCIR329	(AC)8.5	0.63	2	2	0.90	0.47	0.00	0.36
Xcup02	(GCA)6	0.59	6	6	0.96	0.58	0.00	0.53
Xcup53	(TTTA)5	0.79	3	3	1.00	0.34	0.00	0.31
Xcup61	(CAG)7	0.72	6	4	0.98	0.44	0.04	0.41
Xcup63	(GGATGC)4	0.71	6	6	1.00	0.47	0.00	0.43
Xtxp010	(CT)14	0.32	10	10	0.92	0.83	0.00	0.81
Xtxp012	(CT)22	0.52	4	4	0.96	0.61	0.00	0.54
Xtxp015	(TC)16	0.38	7	7	0.98	0.76	0.00	0.73
Xtxp021	(AG)18	0.39	10	9	0.98	0.78	0.02	0.76
Xtxp040	(GGA)7	0.96	3	3	1.00	0.08	0.00	0.08
Xtxp057	(GT)21	0.38	8	8	1.00	0.77	0.00	0.73
Xtxp141	(GA)23	0.25	9	8	1.00	0.83	0.02	0.81
Xtxp145	(AG)22	0.95	3	2	1.00	0.10	0.02	0.09
Xtxp146	(GAA)19	0.98	2	2	1.00	0.04	0.00	0.04
Xtxp320	(AAG)20	0.47	6	6	0.98	0.69	0.00	0.65
Mean	-	0.60	5	5	0.98	0.53	0.01	0.49

numbers of common alleles with frequencies of at least 5%, the observed allele size ranges (bp), as the polymorphic information content (PIC) values (Botstein et al., 1980; Smith et al., 2000) and gene diversity. DARwin v.5.0 (Perrier et al., 2003; Perrier and Jacquemoud-Collet, 2006) was used to calculate pair-wise genetic dissimilarities of accessions using simple matching. The dissimilarity coefficients were used to perform principal coordinates analyses (PCoA) and construct weighted neighbour-joining trees (Saitou and Nei, 1987). Variation between and within accessions was assessed using analysis of molecular variance (AMOVA) using Arlequin v.3.1 (Excoffier et al., 2006).

RESULTS

SSR Polymorphism

Polymorphism among the 48 sorghum genotypes was assessed with 20 SSR markers. All the 20 SSR markers used were polymorphic across the 48 sorghum genotypes with PIC value ranging from 0.04 to 0.81 with a mean of 0.49. Of the 20 markers, 65% were highly polymorphic with a value greater than 0.5 indicating their usefulness in discriminating the genotypes (Table 2). Heterozygosity values of the 20 SSR markers ranged from 0.00 to 0.04, with a mean of 0.01 suggesting that each detected a single genetic locus and that each of the sorghum accession used was stable. The markers revealed a total of 98 alleles with a range between 2 (gpsb067, mSbCIR24 and Xcup53) and 10 (Xtxp320) alleles and an average of 5.05 alleles per primer pair. The gene diversity ranged from 0.04 to 0.83 with a mean value of 0.53. All possible allele combinations found in the 48 accessions ranged from 2 to 10, while the major allele frequency ranged from 0.32 to 0.98 (Table 2).

The weighted neighbour-joining clustering-based dendrogram generated using dissimilarity indices clustered the sorghum accessions into four major groups (Figure 2). Cluster 1 comprised of genotypes from Kitui (west and central), Mbeere (Siakago and Kiritiri), Makueni (Kibwezi, Makindu and Kiboko) and Seredo (commercial variety). The genotypes varied in color from white, brown white, brown, red, black red and purple, but distributed across the four regions. The cluster had three subgroups with genotype black red from mutomo very distinct from other genotypes. Cluster 2 had genotypes from Mbeere (Kiritiri, Siakago), Makueni (Kibwezi), KARI Mtama 1 and Serena commercially released varieties. The cluster had four subgroups with genotypes 7 and 8 from Mbeere Kiritiri grouped together distinctly. Cluster 3 had genotypes from Makueni (Makindu, Kibwezi), Mbeere (Kiritiri, Ishiara, Siakago), Kitui (west and central) and Mutomo; and the commercial variety Gaddam. The cluster had five sub groups with seed color varying greatly. Cluster 4 consists of three genotypes from Mbeere (Kiritiri), Makueni (Kiboko) and Kitui central. Genotype 26 from Mbeere (Kiritiri) and 24 from Kitui central had a closer relationship than with 25, though

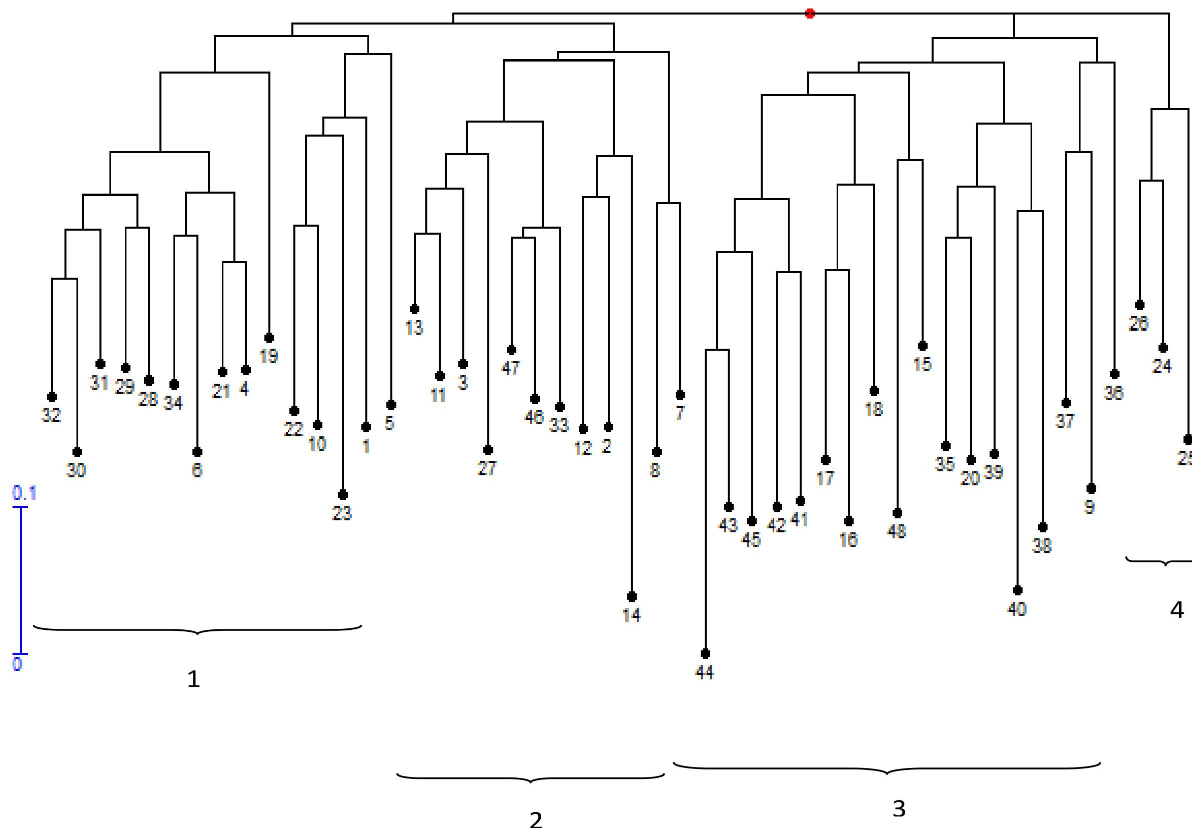

Figure 2. Tree constructed based on 20 polymorphic sorghum SSR markers using the simple matching dissimilarity index and weighted neighbor joining clustering for the 48 sorghum accessions.

in the same cluster (Figure 2).

Genetic diversity among accessions was also confirmed by scatter plot derived through PCoA (Figure 3). Forty percent of the accessions were clustered in the right portions of the plot, while 60% accessions were clustered in the left portion of the plot (axes1/2). Groupings were similar to those detected by cluster analysis where the genotypes were clearly separated across the region except for the genotype 9 from Makueni (Makindu) and genotype 11 from Mbeere (Kiritiri) which had an overlap. Genotypes 20 from Makueni (Kiboko) and 33 from Mbeere (Kiritiri) followed by genotypes 27 from Mbeere (Siakago) and 44 from Kitui central were far much apart from other genotypes; an indication of maximum dissimilarity. Genotypes 11, 13 and 3 formed a solidarity group implying high relatedness in the genetic makeup. This was also observed in genotypes 10 from Makueni (Kibwezi), 22 from Kitui central, 28 from Mutomo, 3 (commercial), 30 from Kiboko and 4 from Mbeere Siakago (Figure 3).

AMOVA showed significant (P = 0.05) differences among the various genotypes evaluated (Table 3). There was a greater variance (91.61%) represented by individuals within populations, while the variance between the groups was less (2.75%) with least variance (1.14%) expressed by the individuals.

Fixation indices between the commercial varieties and genotypes from Mbeere Ishiara was the highest (F_{ST} = 0.24249; P = 0.001), followed by between Ishiara and Kitui central (F_{ST} = 0.19055; P = 0.001). Population pair wise fixation indices was the lowest between Makindu and Kitui west (F_{ST} = 0.03523; P = 0.001) and between Mutomo and Kitui west (F_{ST} = 0.03653; P = 0.001) and Kiritiri Siakago regions (F_{ST} = 0.016; P = 0.001) (Table 4).

DISCUSSION

The genetic diversity among the sorghum accessions used in this study was high as indicated by PIC and gene diversity values. The PIC of a SSR marker gives an idea about the discriminatory power of that marker by taking into account the number of alleles detected and their relative frequencies (Smith et al., 2000). Markers with PIC more than 0.5 are efficient in discriminating genotypes and extremely useful in detecting the polymorphism rate at a particular locus (DeWoody et al., 1995).

Sorghum is primarily an inbreeding species resulting in a low level of observed heterozygosity, but the gene pool as a whole maintains a high level of allelic variation. The

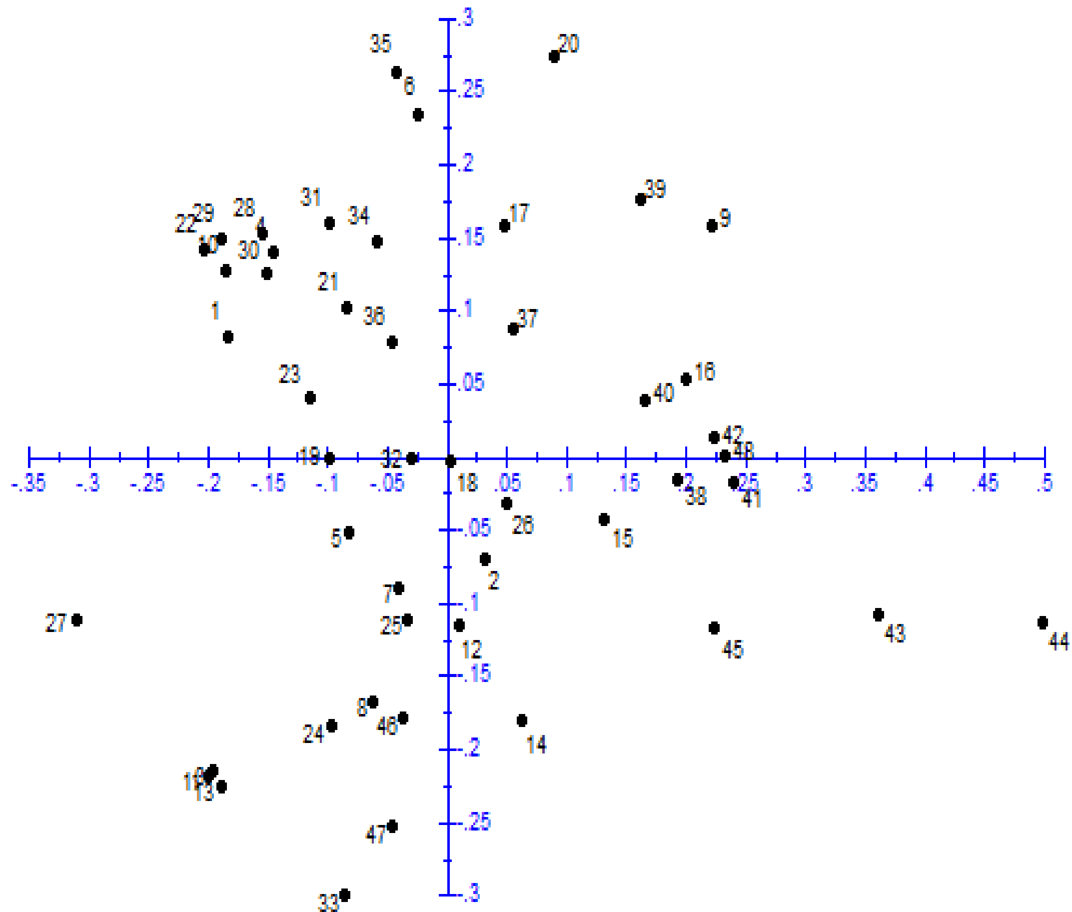

Figure 3. Plot of the axes 1 and 2 of the principal coordinate analysis based on the dissimilarity of 20 SSR markers for 44 sorghum accessions obtained from farmers in lower eastern Kenya and 4 improved varieties.

Table 3. Analysis of molecular variation (AMOVA) of 4 sorghum improved varieties and 44 landraces from farmers in different locations of Mbeere, Mutomo, Kitui and Makueni based on 20 SSR markers.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among populations	9	73.42	0.13	2.75
Among individuals	-	-	-	-
within populations	38	354.10	4.63	91.61
Within individuals	47	2.50	0.05	1.14
Total	94	430.02	4.81	-

high level of allelic variability but low level of heterozygosity observed in this study agrees with findings on diversity analysis of Eritrean sorghum landraces with SSR markers (Ghebru et al., 2002). The low heterozygosity was a clear indication the genotypes were homozygous and thus a high level of stability in the population.

Genetic distances among the 48 genotypes varied greatly indicating a wide diversity. Genotype Karuge 2 and Karuge 1 both from Mbeere (Kiritiri) had a low genetic distance (0.15). Though, the seed color and geographical location of the two accessions was identical, the two are totally different genetically. The results in this study suggest that diversity of the landraces were structured more on geographical locations and on seed colorations than agro-ecological conditions. Reports of other studies in sorghum accessions have shown grouping primarily on the basis of origin, and clustering within groups as driven by racial classification (Sharma

Table 4. Pairwise FST estimates among 4 improved varieties and 44 landraces from different locations in Mbeere, Mutomo, Kitui and Makueni; and 4 improved varieties.

Location	1	2	3	4	5	6	7	8	9	10
Kitui central	-	-	-	-	-	-	-	-	-	-
Kitui west	0.04947	-	-	-	-	-	-	-	-	-
Kiritiri	0.06672	0.07287	-	-	-	-	-	-	-	-
Siakago	0.08234	0.09518	0.07393	-	-	-	-	-	-	-
Ishiara	0.19055	0.06897	0.18737	0.09030	-	-	-	-	-	-
Makindu	0.07124	0.03523	0.05419	0.12299	0.14758	-	-	-	-	-
Kiboko	0.09667	0.11713	0.12840	0.01661	0.14327	0.12209	-	-	-	-
Kibwezi	0.08037	0.07879	0.09231	0.10533	0.18943	0.17455	0.14180	-	-	-
Mutomo	0.05847	0.03653	0.07289	0.07958	0.13146	0.06009	0.07036	0.10138	-	-
Commercial	0.16788	0.16196	0.10243	0.09135	0.24249	0.17748	0.18301	0.15586	0.10933	-

1, Kitui central landrace; 2, Kitui west_landrace; 3, Mbeerekiritiri_landrace; 4, Mbeeresiakago_landrace; 5, Mbeereishiara_landrace; 6, Makindu_landrace; 7, Kiboko_landrace; 8, Kibwezi_landrace; 9, Mutomo_landrace; 10, AS_improved.

et al., 2010; Hash et al., 2007). The main evolutionary forces responsible for producing genetic structure in plant populations are gene flow, selection associated with environmental heterogeneity and/or farmer preferences and random genetic drift (Neal, 2004).

The high genetic diversity could potentially be exploited in broadening the genetic base of germplasm used in sorghum breeding. The unique diversity implies that sorghum landraces are potential source of novel genes, such as pests and disease resistance, low moisture and soil fertility tolerance. The genetic variability and differentiation of the landraces obtained from lower eastern Kenya could be incorporated in breeding programs by developing populations with a broad genetic base. This will help to create new genetic recombinations that can be exploited in response to new breeding challenges.

The clustering of the landraces based on geographical locations was distinct with some overlaps where genotypes cut across the locations. Such intraregional genetic proximity in sorghum landraces would arise through seed exchanges among farmers. This was reported in the baseline survey conducted in sorghum seed systems in Eastern Kenya which were found to be largely traditional, with farmers playing a major role in the selection and exchange of seeds (Muui et al., 2013). A study conducted on sorghum production systems in Bomet, Kenya, indicated that farmers played a great role in seed selection, exchange and movement (Ochieng et al., 2011).

The similarity in genotypes could be attributed to proximity of the regions, though Mbeere appeared to be unique in the color of the seeds. Other studies showed that similarities in genotypes was as a result of region proximity in Africa for cultivated sorghum (Deu et al., 2008; Nkongolo and Nsapato, 2003; Ayana et al., 2000b) and its wild relatives (Ayana et al., 2000a). Results in this study showed that Mbeere had unique cluster. A study in Turkana and North-Eastern Kenya showed that cultivated sorghum was clustered into a distinct and unique genetic group (Mutegi et al., 2010). This is attributed to the fact that Mbeere is relatively geographically remote from other sorghum growing regions. The gene pools from sorghum landraces tested in this study appear to be genetically distinct. A study conducted in Turkana and North Eastern regions of Kenya on cultivated sorghum showed unique genetic clusters based on regions (Mutegi et al., 2010).

AMOVA indicated higher variation within populations than among the groups. Estimates of fixation indices revealed a strong genetic structure between commercial and Mbeere-Ishiara genotypes and between Mbeere-Ishiara and Kitui central genotypes. The presence of strong genetic structure indicates that these two groups are reproductively and genetically isolated from each other.

Conflict of interests

The author(s) did not declare any conflict of interest.

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