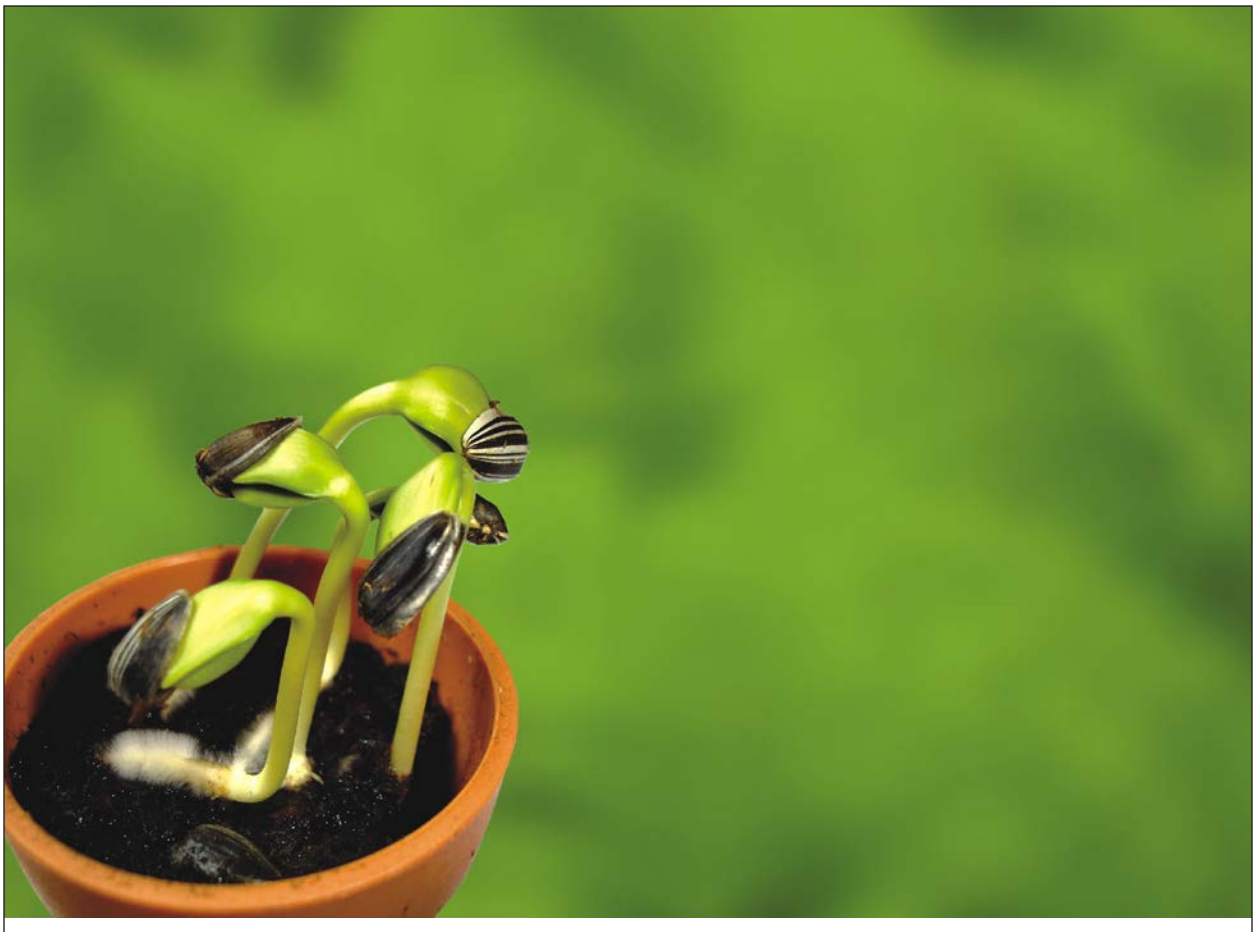

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Research Article
DNA Fingerprinting and Genetic Relationship among Ethiopian Sorghum [*Sorghum bicolor* (L.) Moench] Lines

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Abstract
This study was conducted to develop DNA fingerprint patterns of released sorghum [*Sorghum bicolor* (L.) Moench] varieties in Ethiopia and to assess their genetic relationships. Twelve sorghum lines were genotyped using 39 SSR markers. The SSR analysis showed that 11 of the released lines could be identified by 28 positive and 4 negative unique alleles. E36-1 was identified by seven positive markers, i.e., mSbCIR238, mSbCIR240, Xcup53, Xtxp012, Xtxp145, Xtxp273 and Xtxp320 each having unique allele. B35 was differentiated by four positive; gpsb067, mSbCIR240, Xtxp012, Xtxp015 and two negative markers; Xtxp040 and SbAGB020. Baji could be identified by five positive unique markers; mSbCIR276, Xgap206, Xtxp021, Xtxp141 and Xtxp265 and one negative; Xtxp278 marker. Birmash was identified by four positive markers; Xcup14, Xtxp141, Xtxp145 and Xtxp320. Hormat and Teshale were differentiated with two positive markers each, i.e., Xtxp265, Xtxp320 and gpsb067, Xtxp021, respectively. The other four lines: Abshir, Birhan, Gambella-1107 and Gobye were uniquely identified by one positive marker each; Xtxp265, Xtxp320, mSbCIR238 and Xtxp057, respectively. However, Meko-1 was not uniquely identified by any of the markers used. Genetic dissimilarity among the lines ranged from 0.326-0.839 with an average of 0.672 and the genotypes were grouped into five clusters. The DNA data base generated could be used for proper identification of lines, control of infringement and determine seed mixtures. The information on genetic relationship can be used to select parental lines for crossing programme for development of hybrid sorghum varieties.

Key words: Sorghum, DNA fingerprinting, varietal identification, genetic relationships

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is an important crop in many parts of the world and used as food, feed and industrial purposes. It is a major crop in many parts of Africa and some Asian countries. Sorghum ranked fifth among the cereals produced worldwide after rice, wheat, maize and barley and third in Ethiopia after maize and teff (FAOSTAT., 2011). It is estimated that more than 300 million people especially from developing countries rely on sorghum as source of energy (Godwin and Gray, 2000). Some of the industrial uses of sorghum include preparation of beer, adhesives, dye, resins, ethanol and fuel (House, 1985; NAS., 1996).

In most cases registration and protection of crop varieties rely on morphological traits. The registration involves the documentation and recording of a series of relevant characters (UPOV., 2005) on both candidate and control varieties over at least a two year period. This system, coupled with the fact that discrimination among closely related types is often possible only at later stages of the plant cycle. This makes characterization on the basis of morphological characters difficult, time consuming and ambiguous which results in much interest in the use of molecular markers to complement, assist and/or validate the Distinctiveness Uniformity and Stability (DUS) analysis (De Riek *et al*., 2001; Tommasini *et al*., 2003; Galovic *et al*., 2006).

DNA-based molecular markers provide a solution by providing unique DNA profiles for the protection of new developed varieties, seed purity test and to characterize the varieties. These markers offer high resolving power, high degree of non-tissue specific polymorphism and free of environmental influence (Perry, 2004). The DNA fingerprinting helps to identify and differentiate crop varieties that might be difficult to characterize due to similar morphological characteristics or indistinct traits and to identify plants containing genes of interest such as the confirmation of transformation events (Galovic *et al*., 2006).

Now-a-days, breeders and seed industries use DNA based markers to develop "fingerprint" patterns of their varieties so as to identify the varieties thereby to protect their breeder's rights and to avoid disputes arising from variety ownership claims. However, fingerprinting and genetic relationships among sorghum varieties and lines released for production in Ethiopian has not been done to date. Therefore, this study was initiated to develop a DNA fingerprint pattern of released lines in and to assess their genetic relationship thereby the information generated could be used by breeders and seed companies to distinguish the released varieties and choose potential parental lines for crossing to develop hybrid varieties.

MATERIALS AND METHODS

Plant materials: The study consists of 12 sorghum lines provided by Melkassa and Sirinka Agricultural Research Centers. The list of released lines is presented in Table 1.

SSR markers: Thirty nine Simple Sequence Repeats (SSRs) were used in the study, including 22 di, 9 tri and 4 tetra nucleotide or longer motifs and 4 compound repeats. These SSR markers were selected based on their uniform distribution in the sorghum genome. These are the same set of markers that were selected and used by the Generation Challenge Programme for genetic diversity assessment of global sorghum germplasm. List of the SSR markers, including primer sequences, information on repeat motif and length are given in Table 2.

Table 1: List of sorghum lines used in the DNA fingerprinting study

Released line	Pedigree/source	Adaptation	Year of release	Special merit
Abshir	P9403 (Purdue university)	Low land	2000	Striga resistant
Gobye	P9401 (Purdue university)	Low land	2000	Striga resistant
E36-1	ICRISAT	Low land	NA	Stay-green
B35	ICRISAT	Low land	NA	Stay-green
Meko-1	M36121 (ICRISAT)	Low land	1997	Early maturing
Teshale	3443-1-OP (ICRISAT)	Low land	2002	Early maturing
Gambella-1107	Selected from landraces	Low land	1976	Medium to early maturing
76T1-23	954062 x 73pp9	Low land	1979	Extraearly maturing
Baji	85 MW 5334 (RS/R-20-8614-2 x IS9293)	Mid alt.	1996	High yield
Birmash	80 LPYT-1 #433 x IS 9302	Mid alt.	1989	High yield
Hormat	ICSV 1112 BF (ICRISAT)	Low land	2005	Striga resistant
Birhan	PSL5061(Purdue university)	Low land	2002	Striga resistant

*NA: Information not available

Table 2: List of SSR markers, primer sequences, repeat motif and annealing temperatures

Marker name	Repeat motif	Forward primer	Reverse primer	Ann. T° (°C)
Gpsb067	(GT)10	TAG TCC ATA CAC CTT TCA	TCT CTC ACA CAC ATT CTTC	49
Gpsb123	(CA)7+(GA)5	ATA GAT GTT GAC GAA GCA	GTG GTA TGG GAC TGG A	50
Xisep0310	(CCAAT)4	TGC CTT GTG CCT TGT TTA TCT	GGA TCG ATG CCT ATC TCG TC	60
mSbCIR223	(AC)6	CGT TCC AAT GAC TTT TCT TC	GCC AAT GTG GTG TGA TAA AT	55
mSbCIR238	(AC)26	AGA AGA AAA GGG GTA AGA GC	CGA GAA ACA ATT ACA TGA ACC	55
mSbCIR240	(TG)9	GTT CTT GGC CCT ACT GAA T	TCA CCT GTA ACC CTG TCT TC	55
mSbCIR246	(CA)7.5	TTT TGT TGC ACT TTT GAG C	GAT GAT AGC GAC CAC AAA TC	55
mSbCIR248	(GT)7.5	GTT GGT CAG TGG TGG ATA AA	ACT CCC ATG TGC TGA ATC T	56
mSbCIR262	(CATG)3.25	GCA CCA AAA TCA GCG TCT	CCA TTT ACC CGT GGA TTA GT	57
mSbCIR276	(AC)9	CCC CAA TCT AAC TAT TTG GT	GAG GCT GAG ATG CTC TGT	53
mSbCIR283	(CT)8 (GT)8.5	TCC CTT CTG AGC TTG TAA AT	CAA GTC ACT ACC AAA TGC AC	54
mSbCIR286	(AC)9	GCT TCT ATA CTC CCC TCC AC	TTT ATG GTA GGA TGC TCT GC	55
mSbCIR300	(GT)9	TTG AGA GCG GCG AGG TAA	AAA AGC CCA AGT CTC AGT GCT A	61
mSbCIR306	(GT)7	ATA CTC TCG TAC TCG GCT CA	GCC ACT CTT TAC TTT TCT TCT G	55
mSbCIR329	(AC)8.5	GCA GAA CAT CAC TCA AAG AA	TAC CTA AGG CAG GGA TTG	54
SbAGB02	(AG)35	CTC TGA TAT GTC GTT GTG CT	ATAGAGAGGATAGCTTATAGCTCA	55
Xcup002	(GCA)6	GAC GCA GCT TTG CTC CTA TC	GTC CAA CCA ACC CAC GTA TC	54
Xcup014	(AG)10	TAC ATC ACA GCA GGG ACA GG	CTG GAA AGC CGA GCA GTA TG	54
Xcup053	(TTTA)5	GCA GGA GTA TAG GCA GAG GC	CGA CAT GAC AAG CTC AAA CG	54
Xcup061	(CAG)7	TTA GCA TGT CCA CCA CAA CC	AAA GCA ACT CGT CTG ATC CC	54
Xcup063	(GGATGC)4	GTA AAG GGC AAG GCA ACA AG	GCC CTA CAA AAT CTG CAA GC	54
Xgap072	(AG)16	TGCACCAC TCT GGA AAA GGC TA	CTGAGGACTGCCCCAAATGTAGG	55
Xgap084	(AG)14	CGC TCT CGG GAT GAA TGA	TAACGGACCACTAACAAATGATT	55
Xgap206	(AC)13/(AG)20	ATTCATCATCCTCATCCTCGTAGAA	AAA AAC CAA CCC GAC CCA CTC	55
Xtxp010	(CT)14	ATA CTA TCA AGA GGG GAG C	AGT ACT AGC CAC ACG TCA C	50
Xtxp012	(CT)22	AGA TCT GGC GGC AAC G	AGT CAC CCA TCG ATC ATC	55
Xtxp015	(TC)16	CAC AAA CAC TAG TGC CTT ATC	CAT AGA CAC CTA GGC CAT C	55
Xtxp021	(AG)18	GAG CTG CCA TAG ATT TGG TCG	ACC TCG TCC CAC CTT TGT TG	60
Xtxp040	(GGA)7	CAG CAA CTT GCA CTT GTC	GGG AGC AAT TTG GCA CTA G	55
Xtxp057	(GT)21	GGA ACT TTT GAC GGG TAG TGC	CGA TCG TGA TGT CCC AAT C	55
Xtxp114	(AGG)8	CGT CTT CTA CCG CGT CCT	CAT AAT CCC ACT CAA CAA TCC	50
Xtxp136	(GCA)5	GCG AAT AGC ATC TTA CAA CA	ACT GAT CAT TGG CAG GAC	55
Xtxp141	(GA)23	TGT ATG GCC TAG CTT ATC T	CAA CAA GCC AAC CTA AA	55
Xtxp145	(AG)22	GTT CCT CCT GCC ATT ACT	CTT CCG CAC ATC CAC	55
Xtxp265	(GAA)19	GTC TAC AGG CGT GCA AAT AAA A	TTACCATGCTACCCCTAAAAGTGG	55
Xtxp273	(TTG)20	GTA CCC ATT TAA ATT GTT TGC AGT AG	CAG AGG AGG AGG AAG AGA AGG	55
Xtxp278	(TTG)12	GGG TTT CAA CTC TAG CCT ACC GAA CTT CCT	ATGCCTCATCATGGTTCGTTTTGCTT	50
Xtxp320	(AAG)20	TAA ACT AGA CCA TAT ACT GCC ATG ATAA	GTGCAAATAAGGGCTAGAGTGTT	54
Xtxp321	(GT)4+(AT)6+(CT)21	TAA CCC AAG CCT GAG CAT AAG A	CCC ATT CAC ACA TGA GAC GAG	55

DNA extraction: Seedlings were grown in the greenhouse during November, 2010. Fresh leaves of 10 individual plants were harvested in bulk from 14 days old seedlings and dried with silica gel in zip locked plastic bags and used for DNA extraction. The DNA was extracted following a modified CTAB (Cetyl Trimethyl Ammonium Bromide) extraction protocol (Mace *et al*., 2003) at BecA Laboratory, Nairobi, Kenya. A labeled 96 well tube box that contained one stainless steel grinding ball in each tube were put in ice bucket containing liquid nitrogen to chill the tubes.

Approximately 1.2 cm^2 sorghum leaves were placed into 96 well strip tubes and slid with forceps to about 5 mm above the steel ball strip, caps were put tightly on the tubes, a third-folded paper towel on top of them and covered with a lid and stored at -20°C until ready to grind. Caps were removed carefully and about 450 µL of preheated (65°C) extraction buffer (100 mM Tris-HCl [pH 8], 1.4 M NaCl, 20 mM EDTA, 3% CTAB and 0.17% β-mercaptoethanol) was added to each sample and closed with caps. Samples were then macerated using a Geno/grinder (Geno 2000, Sigma) at 500 strokes/min for 2 min. The macerated samples were incubated for 40 min at 65°C in water bath with occasional mixing. Solvent extraction was done by adding 450 µL chloroform: isoamylalcohol (24:1) to each tube and inverted twice to mix. The tubes were centrifuged at 3500 rpm for 15 min and the entire upper aqueous layer was transferred to fresh strip tubes. About 500 µL of pre-cooled isopropanol (stored at -20°C) was added and inverted three times to mix and kept in -20°C freezer for 2 h. Then the tubes were centrifuged at 3500 rpm for 30-35 min. The supernatant was then decanted, the pellet was air dried for 30 min and dissolved in 200 µL low salt Tris-EDTA (TE) buffer (10 mM Tris,

0.1 mM EDTA, pH 8). To remove RNA from the DNA solution, 3 µL RNase A (10 mg mL^{-1}) was added to each sample and incubated for 30 min at 37°C. A second phase solvent extraction was done by adding 200 µL chloroform: isoamyl alcohol (24:1) to each sample and inverted twice to mix and centrifuged at 3500 rpm for 15 min.

The upper layer was transferred to new strip tubes and 500 µL ethanol: sodium acetate solution was added to each sample, then inverted twice to mix and placed in -20°C for 2 h and then centrifuged at 3,500 rpm for 30 min. The supernatant was decanted and the pellets washed with 200 µL 70% precooled ethanol. The tubes were centrifuged at 3,500 rpm for 5 min, the supernatant decanted and the pellet air-dried for about an hour. Finally, pellets from each sample were dissolved in 100 µL low-salt TE buffer and stored at -20°C.

Determination of DNA quality and concentration: The quality and quantity of the isolated DNA was determined by comparing the fluorescence of aliquots of DNA samples with a known concentration of l-DNA after running them on 0.8% agarose gel (8 g agarose dissolved in100 mL 1×TBE) buffer that contained 0.3 mg mL^{-1} ethidium bromide solution. For this purpose, samples were prepared by mixing 3 mL of the DNA solution, 3 mL loading dye and 4 mL distilled water and loaded on the gel and run at 120 volts for 30 min in 1×TBE buffer. At the end of electrophoresis, the gel was visualized using UV light and photographed using a video capture (Flowgen IS 1000). All samples were normalized to the same concentration level and used for PCR.

Polymerase chain reaction conditions and amplifications: The PCR was performed using Gene-Amp PCR System 9600 (PE-Applied Biosystems) in 96 well plates in a total reaction volume of 10 µL that consisted of 1 µL DNA, 1 µL PCR buffer, 2 µL $MgCl_2$, 1.0 µL reverse primer, 1.0 µL forward primer directly labelled with 6-FAM (VIC, NED, PET fluorescent dyes), 0.5 µL of each dNTP, 0.04 µL Taq DNA polymerase and 3.46 H_2O. The amplification profile consisted of initial denaturation of the template DNA at 95°C for 3 min, followed by 35 cycles, each for 30 sec at 95°C (denaturation), 1 min at 56°C (annealing) and 1 min at 72°C (extension) and a final extension at 72°C for 3 min.

Capillary electrophoresis: After the PCR, a small amount of samples from each primer pair product were randomly selected and checked for proper amplification and product intensity on 2% agarose gel and an ABI plate was prepared with a total volume of 10 µL (9.0 µL from a mix of an injection solution (1 mL formamide and 12 µL GS500 LIZ (Perkin Elmer-Applied Biosystems) for 96 well platesand 1.0 µL of PCR products from each of the 6-FAM, VIC, NED and PET-labelled PCR products were pooled together).

The DNA fragments were denatured at 95°C for 3 mins, chilled quickly and size-fractioned using ABI 3730 capillary DNA Sequencer (Perkin Elmer-Applied Biosystems). In this system, the labeled PCR products were detected using a laser and capillary electrophoresis based on their fluorescent dye and fragment size. The peaks were sized and the alleles mapped using Gene Mapper software version 3.7 (Perkin Elmer-Applied Biosystems) and presented as alleles scored as estimated fragment sizes in base pairs compared to the internal size standard GS500LIZ-3730.

Data analysis: The pair-wise Genetic Similarity (GS) matrix was calculated based on Jaccard's similarity coefficient (Jaccard, 1908): MSij = Nij/(Nii+Nij+Njj), where MSij is the DNA marker similarity index between the ith and jth genotype, Nij is the number of bands present in both genotypes Nii is the number of bands present in the ith genotype but lacking in the jth genotype and Njj is the number of bands lacking in the ith genotype but present in the jth genotype.

Clustering was done by an agglomerative hierarchical classification (Rohlf, 1992), employing unweighed pair group method using arithmetic averages (UPGMA). To test the goodness of fit of clustering to the similarity matrix, co-phenetic correlation (r) was calculated using the equation:

$$r = (\Sigma Xi\ Yi - \Sigma Xi\ \Sigma Yi/n)/SXi\ SYi$$

where, Xi and Yi are the similarity or distance values of the original and cophenetic matrix, respectively. The SXi and SYi are the standard deviations for each variable. Data analysis was doneusing Darwin5 statistical package version 5.0.158.

RESULTS AND DISCUSSION

Identification of varieties using unique markers: Unique markers are defined as bands that specifically identify varieties from the others by their presence or absence. As previously mentioned by El-Awady *et al.* (2008), alleles that are present in one variety but not found in the others are termed Positive Unique Markers (PUM), whereas a Negative Unique Markers (NUM) is the opposite of positive markers. In the present study, it was possible to differentiate the 11 released lines using SSR markers. Twenty one SSRs out of the 39 revealed 32 unique alleles (28 positive and 4 negative) (Table 3).

Table 3: Unique positive and negative SSR alleles with their sizes for Ethiopian released sorghum lines

	Positive unique marker		Negative unique marker	
Released line	Marker	Allele size (bp)	Marker	Allele size (bp)
76T1-23	-	-	mSbCIR246	100
Abshir	Xtxp25	207	-	-
B35	gpsb067	172	Xtxp040	134
	mSbCIR240	159	SbAGB02	95
	Xtxp012	185		
	Xtxp015	221		
Baji	mSbCIR276	229	Xtxp278	248
	Xgap206	139		
	Xtxp021	187		
	Xtxp141	163		
	Xtxp265	175		
Birhan	Xtxp320	260	-	-
Birmash	Xcup14	234	-	-
	Xtxp141	155		
	Xtxp145	242		
	Xtxp320	224		
E36-1	mSbCIR238	83	-	-
	mSbCIR240	107		
	Xcup53	186		
	Xtxp012	173		
	Xtxp145	244		
	Xtxp273	223		
	Xtxp320	263		
Gambella-1107	mSbCIR238	87	-	-
Gobye	Xtxp057	253	-	-
Hormat	Xtxp265	201	-	-
	Xtxp320	278		
Teshale	gpsb067	184	-	-
	Xtxp021	177		

-: Absent

The E36-1 was identified by seven markers, i.e., mSbCIR238, mSbCIR240, Xcup53, Xtxp012, Xtxp145, Xtxp273 and Xtxp320 each having unique allele. B35 was differentiated by four PUM, i.e., gpsb067, mSbCIR240, Xtxp012, Xtxp015 and two NUM, i.e., Xtxp040 and SbAGB020. Baji could be identified by five positive unique markers (mSbCIR276, Xgap206, Xtxp021, Xtxp141 and Xtxp265) and one negative (Xtxp278). Birmash was identified by four positive markers (Xcup14, Xtxp141, Xtxp145 and Xtxp320). Hormat and Teshale were differentiated with two positive markers each, i.e., Xtxp265, Xtxp320 and gpsb067, Xtxp021, respectively. The other four lines-Abshir, Birhan, Gambella-1107 and Gobye were uniquely identified by one positive marker each Xtxp265, Xtxp320, mSbCIR238 and Xtxp057, respectively. However, Meko-1 was not uniquely identified by any of the markers used.

As indicated in Table 3 more unique alleles were produced by Xtxp320 which alone uniquely distinguishes four of the 12 released lines, namely E36-1, Birhan, Hormat and Birmash. In Xgap206, nine alleles were scored, however, the number of unique allele is only one which uniquely identified only Baji.

El-Awady *et al*. (2008) reported identification of four out of nine *Sorghum bicolor* genotypes with nine SSR markers. According to Bandelj *et al*. (2002), a minimum of three microsatellite markers were found sufficient for rapid and unambiguous discrimination of olive varieties. In another study by Olufowote *et al*. (1997), as few as six, well-chosen SSLPs were sufficient to discriminate between 71 related lines of rice. A study conducted in Bangladesh discriminated 26 rice cultivars out of 34 using three SSR markers (Rahman *et al*., 2009). Sarao *et al*. (2009) differentiated genotypes of basmati rice from the non-basmati rice using four markers. Similarly, Chakravarthi and Naravaneni (2006) uniquely identified nine rice genotypes out of 15. They stated that fingerprinting makes identification and characterization of genotypes easy and it further helps in background selections during back-cross breeding programs. Kwon *et al*. (2005) studied fingerprinting of pepper and distinguished 60 of 66 varieties using 27 polymorphic SSR markers.

The SSR markers and their respective unique alleles could have a number of potential applications including the determination of cultivar purity, varietal identification, varietal ownership dispute resolution and other similar applications. The present study attempted to find out a set of microsatellite markers to differentiate lines released in Ethiopia, providing meaningful data that can be used by sorghum breeders and seed companies in distinguishing the lines.

Genetic relationships among lines: To determine the genetic relationships among the 12 lines based on the SSR data generated, Jaccard's similarity coefficient was employed. Genetic dissimilarity among the lines ranged from 0.326-0.839 with an average of 0.672. The highest dissimilarity was observed between B35 and Teshale (0.839) followed by B35 and Gobye (0.833). The lowest dissimilarity was observed between Meko-1 and Teshale (0.326) followed by Meko-1 and Gambella-1107 (0.348).

The dissimilarity coefficients (Table 4) were used to produce an agglomerative hierarchical classification by employing unweighted pair group method using arithmetic averages (UPGMA). The dendrogram consisted of five clusters (Fig. 1). The first cluster contains Baji and Birmash. The second contains Birhan, Gobye and Abshir. The third cluster contains three sub groups. The first subgroup contains Hormat and Gambella-1107, the second Meko-1 and Teshale and the third 76T1-23. B35, the known stay-green source genotype obtained from the Zera Zera line of Ethiopia was put in a separate cluster which indicates that it has a wide genetic distance from the other varieties. Similarly, E36-1, the other stay green source originated in Ethiopia was assigned in a separate cluster.

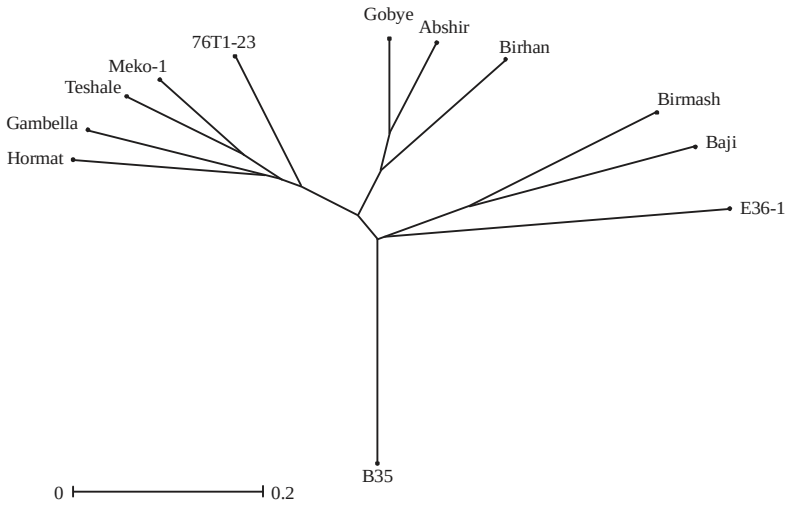

Fig. 1: Genetic relationship among 12 sorghum released lines

Table 4: Pairwise genetic dissimilarity among 12 sorghum varieties based on Jaccard similarity coefficient using 39 SSR markers

Released line	76T1-23	Abshir	B35	Baji	Birhan	Birmash	E36-1	Gambella-1107	Gobye	Hormat	Meko-1
Abshir	0.61										
B35	0.76	0.78									
Baji	0.81	0.78	0.81								
Birhan	0.71	0.48	0.75	0.63							
Birmash	0.82	0.81	0.79	0.53	0.70						
E36-1	0.78	0.72	0.74	0.75	0.75	0.71					
Gambella-1107	0.42	0.66	0.81	0.78	0.72	0.80	0.73				
Gobye	0.67	0.35	0.83	0.77	0.50	0.79	0.77	0.69			
Hormat	0.52	0.64	0.77	0.72	0.63	0.68	0.66	0.40	0.64		
Meko-1	0.44	0.61	0.80	0.77	0.65	0.80	0.73	0.35	0.65	0.44	
Teshale	0.62	0.65	0.84	0.74	0.63	0.78	0.74	0.48	0.66	0.50	0.33

Although, pedigree data was not available for each line used in this study, the grouping of the lines could be based on their pedigree relationship or similarity in their source breeding materials. Meko-1 and Teshale, the most similar released lines were introduced from ICRISAT which might have similar source material. Birhan, Gobye and Abshir grouped in the first cluster were introduced from Purdue University which might have also the same source material.

The high cophenetic correlation (r = 0.97) observed in the present study between the dissimilarity matrix and the dendrogram is an indicative of a good representation of the plot to the dissimilarities. Similar studies by Chakauya *et al*. (2006) reported less values for cophenetic correlation (r = 0.71). The results of the present study indicated that there is a wide genetic dissimilarity among the studied lines. Hallauer and Miranda (1988) reported that the genetic distance information is useful to breeders for planning crosses, in assigning lines to specific heterotic groups and for precise identification with respect to plant varietal protection. Studies of genetic diversity and genetic relatedness assisted by molecular markers can improve the use of the different genotypes in breeding programs and the design of new crosses.

REFERENCES

Bandelj, D., J. Jakse and B. Javornik, 2002. DNA fingerprinting of olive varieties by microsatellite markers. Food Technol. Biotechnol., 40: 185-190.

Chakauya, E., P. Tongoona, E.A. Matibiri and M. Grum, 2006. Genetic diversity assessment of sorghum landraces in Zimbabwe using microsatellites and indigenous local names. Int. J. Bot., 2: 29-35.

Chakravarthi, B.K. and R. Naravaneni, 2006. SSR marker based DNA fingerprinting and diversity study in rice (*Oryza sativa* L.). Afr. J. Biotechnol., 5: 684-688.

De Riek, J., E. Calsyn, I. Everaert, E. Van Bockstaele and M. De Loose, 2001. AFLP based alternatives for the assessment of distinctness, uniformity and stability of sugar beet varieties. Theoret. Applied Genet., 103: 1254-1265.

El-Awady, M., S.S. Youssef, E.E.M. Selim and M.M. Ghonaim, 2008. Genetic diversity among *Sorghum bicolor* genotypes using Simple Sequence Repeats (SSRs) markers. Arab. J. Biotechnol., 11: 181-192.

FAOSTAT., 2011. FAO statistical data base for food. Food and Agriculture Organization of the United Nations, Rome, Italy.

Galovic, V., S. Mladenovic-Drinic, J. Navalusic and M. Zlokolica, 2006. Characterization methods and fingerprinting of agronomical important crop species. Genetika, 38: 83-96.

Godwin, I.D. and S.J. Gray, 2000. Overcoming Productivity and Quality Constraits in Sorghum: The Role for Genetic Engineering. In: Transgenic Cereals, O'Brien, L. and R.J. Henry (Eds.). American Association of Cereal Chemists, St. Paul, MN., pp: 153-177.

Hallauer, A.R. and J.B. Miranda, 1988. Quantitative Genetics in Maize Breeding. 2nd Edn., Iowa State University Press, Ames.

House, L.R., 1985. A Guide to Sorghum Breeding. 2nd Edn., ICRISAT Patanchru, India.

Jaccard, P., 1908. Nouvelles researches surla distribution florale. Bull. Soc. Vaud. Sci. Nat., 44: 223-270.

Kwon, Y.S., J.M. Lee, G.B. Yi, S.I. Yi and K.M. Kim *et al*., 2005. Use of SSR markers to complement tests of distinctiveness, uniformity and stability (DUS) of pepper (*Capsicum annuum* L.) varieties. Mol. Cells, 19: 428-435.

Mace, E.S., K.K. Buhariwalla, H.K. Buhariwalla and J.H. Crouch, 2003. A high-throughput DNA extraction protocol for tropical molecular breeding programs. Plant Mol. Biol. Rep., 21: 459-460.

NAS, 1996. Lost Crops of Africa: Grains. National Academy Press, Washington, DC.

Olufowote, J.O., Y. Xu, X. Chen, M. Goto and S.R. McCouch *et al*., 1997. Comparative evaluation of within-cultivar variation of rice (*Oryza sativa* L.) using microsatellite and RFLP markers. Genome, 40: 370-378.

Perry, D.J., 2004. Identification of Canadian durum wheat varieties using a single PCR. Theoret. Applied Genet., 109: 55-61.

Rahman, M.S., M.R. Molla, M.S. Alam and L. Rahman, 2009. DNA fingerprinting of rice (*Oryza sativa* L.) cultivars using microsatellite markers. Aust. J. Crop Sci., 3: 122-128.

Rohlf, F.J., 1992. NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System. Version 1.70, Exeter Software, Setauket, New York.

Sarao, N.K., Y. Vikal, K. Singh, M.A. Joshi and R.C. Sharma, 2010. SSR marker-based DNA fingerprinting and cultivar identification of rice (*Oryza sativa* L.) in Punjab state of India. Plant Genet. Resour., 8: 42-44.

Tommasini, L., J. Batley, G.M. Arnold, R.J. Cooke and P. Donini *et al*., 2003. The development of multiplex Simple Sequence Repeat (SSR) markers to complement distinctness, uniformity and stability testing of rape (*Brassica napus* L.) varieties. Theoret. Applied Genet., 106: 1091-1101.

UPOV., 2005. Meeting on enforcement of plant breeders' rights. International Union for the Protection of New Varieties of Plants, Geneva, Switzerland.