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Full Length Research Paper

Assessment of genetic diversity in sorghum accessions using amplified fragment length polymorphism (AFLP) analysis

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Amplified fragment length polymorphism (AFLP) analysis was used to assess the genetic relationships among 46 accessions of sorghum (*Sorghum bicolor* (L.) Moench) collected from the north-western, western and central parts of Ethiopia as well as South Africa. Six AFLP primer combinations were used for the analysis of DNA fragment amplification. Dice similarity coefficients were calculated and a dendrogram was constructed following the UPGMA method of cluster analysis. A total of 186 fragments were amplified of which 78 (43.10%) were polymorphic. The number of polymorphic fragments amplified per primer combination varied from 9 to 21. Genetic polymorphism present among sorghum accessions was low, as evidenced by the high level of similarity in the AFLP marker profiles of different sorghum accessions. Pair-wise genetic similarity coefficients ranged from 0.87 to 0.99, with an average of 0.92. This indicates low levels of genetic diversity among tested sorghum accessions. The landraces were genetically very similar, while the differences between landraces, the Ethiopian cultivars and the South African cultivars were relatively higher. Genetic similarity within the South African and the Ethiopian cultivars was very high. Almost all accessions clustered according to the geographical origin. Results of this study indicate that the landraces were related, and this most likely results from the exchange of seeds between farmers in the collection regions, although no duplications were found in the material.

Key words: Amplified fragment length polymorphism (AFLP), accessions, genetic diversity, sorghum.

INTRODUCTION

The eastern African region is considered as one of the centres of genetic diversity (Vavilov, 1926) and area of domestication for sorghum (Doggett, 1965). Studies using molecular markers have supported and confirmed that the central and north-eastern regions of Africa were the main areas of sorghum domestication (Deu et al., 1994).

Assessment of sorghum genetic diversity and the relationship among and within sorghum accessions are crucial for sorghum improvement programmes in Africa (Dean et al., 1999; Abu Assar et al., 2005; 2009) and high levels of genetic variation were reported in Eastern African region, particularly in Ethiopia (Ayana and Bekele, 1998, 1999, 2000). A greater scope for genetic diversity among both cultivated and wild sorghum was found in Ethiopia (Doggett, 1988).

DNA markers have been applied to assess and characterise genetic diversity within and among crop species and these will help in identifying important sources of genetic variability (Menz et al., 2004; Todorovska et al., 2005; Kumar et al., 2008). Genetic erosion resulted in loss of biodiversity which has

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emphasised the importance of characterising germplasm accessions, including local landraces and other plant materials for genetic resource conservation (Food and Agriculture Organization (FAO), 1998; Todorovska et al., 2005). Hence, consideration must be given to evaluate genetic resources such as landraces as well as breeding material at DNA level for characterization, evaluation, maintenance and improvement of genetic diversity (Todorovska et al., 2005).

Conservation of genetic diversity within and among a species is very important to achieve genetic gain towards targeted goals and food security (Gray, 1996). Conventional plant breeding is time consuming and highly dependent on environmental conditions. The application of molecular markers is crucial and more effective and efficient for selection of traits of interest in breeding programmes as well as to assess genetic diversity, since these markers are not influenced by environment (Fufa et al., 2005; Geleta et al., 2006; Shehzad et al., 2009). Molecular markers play a major role in the determination of genetic variability and relationships and different kinds of markers have been used in many studies of sorghum (Shehzad et al., 2009) for example restriction fragment length polymorphism (RFLPs) (Ahnert et al., 1996), random amplified polymorphic DNA (RAPDs) (Iqbal et al., 2010), simple sequence repeats (SSRs) (Bucheyeki et al., 2009) and amplified fragment length polymorphism (AFLPs) (Menz et al., 2004; Ritter et al., 2007).

These molecular markers have been used efficienlty to characterise and estimate the genetic diversity among sorghum germplasm. Therefore, the present study was designed to assess the biodiversity of sorghum accessions from the north-western, western and central parts of Ethiopia as well as from South Africa, using AFLP markers.

MATERIALS AND METHODS

The sorghum accessions used in this study were obtained from the Institute of Biodiversity Conservation (IBC)/Ethiopia and were collected from the north-western, western and central parts of Ethiopia. Ten accessions from South Africa were included for comparison purposes (Table 1).

The leaf material was obtained from the three week-old plants of each sorghum accessions. Young harvested leaves were freeze-dried and then ground to fine powder using a QiagenTissueLyser. The total genomic DNA was isolated using hexadecyltrimethylammonium bromide (CTAB) method (Saghai-Maroof et al., 1984). A volume of 750 µl CTAB buffer [100 mMtrishydroxymethylaminomethane, pH 8.0); 20 mM EDTA (ethylene-diaminetetra acetate), pH 8.0; 1.4 M NaCl; 2% (w/v) CTAB; 0.2% (v/v) β-Mercaptho-ethanol] was added to approximately 250 µl fine leaf powder in a 1.5 ml microfuge tube and incubated in a water bath at 65°C for 1 h. The suspension was extracted with 500 µl chloroform: isoamylalcohol [24:1 (v/v)] and the phases were separated by centrifugation at 12000 *g* for 3 min. DNA was precipitated from the aqueous phase with 0.66 volumes isopropanol at room temperature for 20 min and centrifuged at 12000 *g* for 10 min.

DNA isolation

The precipitate was washed at room temperature with 500 µl 70% (v/v) ethanol for 20 min followed by centrifugation at 12000 *g* for 5 min. The pellet was air-dried for 1 h and re-suspended in TE buffer (10 mM trishydroxymethylaminomethane, pH 8.0; 1 mM EDTA, pH 8.0). Re-suspended DNA was precipitated with 0.75 M ammonium acetate and equal volume chloroform: isoamylalcohol [24:1 (v/v)].

DNA was precipitated from the aqueous layer with two volumes of ice-cold absolute ethanol. After an overnight incubation at -20°C, DNA was recovered by centrifugation at 12000 *g* for 15 min and washed twice with ice-cold 70% (v/v) ethanol for 5 min. The pellet was air-dried and resuspended in TE buffer and treated with 0.1 µg μl^{-1}DNase-free RNase for 2 h at 37°C. DNA quantity and quality were estimated using a UV spectrophotometer by measuring absorbances at A_{260} and A_{280}. DNA samples were diluted to a working solution of 200 ngμl^{-1}.

Amplified fragment length polymorphism (AFLP) analysis

AFLP analysis was performed using six primer pair combinations (Table 2). *Mse*I-primers were screened in combination with *Eco*RI-primers (*EcoRI* and *Mse*I primers) and were given names beginning with E and M, respectively. The code following E or M refers to the three selective nucleotides at the 3'-end of the primer. This coding system was used throughout. Primers and adapters were synthesized by Integrated DNA Technologies Inc. (Coralville, USA). Oligonucleotides used for adapters were polyacrylamide gel electrophoresis (PAGE) purified. Adapters were prepared by adding equimolar amounts of both strands, heating for 10 min to 65°C in a water bath and then leaving the mixture to cool down to room temperature. AFLP analysis were performed as described by Vos et al. (1995) and modified by Herselman (2003). Genomic DNA (±1.0 µg) was digested using 4 U of *Mse*I (New England Prolabs) and 1x *Mse*I-buffer [50 mM NaCl; 10 mM trishydroxymethylaminomethane, pH 7.9; 10 mM $MgCl_2$; 0.1 mM DTT (dithiotreitol)] in a final volume of 50 µl for 5 h at 37°C. Following *Mse*I digestion, DNA was further digested overnight at 37°C with 5U *Eco*RI and NaCl to a final concentration of 100 mM. Adapter ligation of the digested DNA was obtained by adding a solution containing 50 pmol*Mse*I-adapter, 5 pmol*Eco*RI-adapter, 1 U T4 DNA Ligase (USB Corporation), 0.4 mM ATP (adenosinetriphosphate) and 1 × T4 DNA ligase buffer (66 mM trishydroxymethylaminomethane, pH 7.6; 6.6 mM $MgCl_2$; 10 mM DTT; 66 mM ATP) followed by overnight incubation at 16°C.

Pre-amplification reactions

Pre-amplification reactions were carried out in 50 µl reaction mixtures containing 5 µl template DNA (restriction/ligation mixture), 30 ng of each pre-amplification primer (*Eco*RI- and *Mse*I-primer+1) (Table 2), 1x Promega*Taq* polymerase buffer (10 mM trishydroxymethylaminomethane, pH 9.0; 50 mM KCl; 0.1% (v/v) Trition x-100), 2 mM $MgCl_2$, 200 µM of each dNTP and 1 U *Taq* DNA polymerase (Promega, Madison, WI, USA). Amplifications were performed using the following cycling programme: 5 min at 94°C, 30 cycles of 30 s at 94°C, 60 s at 56°C and 60 s at 72°C and a final extension of 10 min at 72°C. Quality and quantity of pre-amplification reactions were determined by electrophoresis in 1.5% (w/v) agarose gels and diluted accordingly (1:5 to 1:15 times) prior to selective amplification.

Selective amplification

Selective amplification reactions were performed in a total volume of 20 µl reaction containing 5 µl of diluted pre-amplification product,

Table 1. List of sorghum accessions, collection sites and the status of accessions used for AFLP marker analysis.

Number	Acc. No./Name*	Major agroecology	Region	Zone	District	Adaptation zone	Status
1	69029	North-west	BGR	Metekel	Dibate	Lowland	Landrace
2	69030	North-west	BGR	Metekel	Dibate	Lowland	Landrace
3	69032	North-west	BGR	Metekel	Dangur	Lowland	Landrace
4	69128	West	BGR	Assosa	Assosa	Lowland	Landrace
5	69147	West	BGR	Metekel	Wenbera	Intermediate	Landrace
6	69164	West	BGR	Metekel	Wenbera	Lowland	Landrace
7	69165	West	BGR	Metekel	Dangur	Intermediate	Landrace
8	69538	West	Oromya	Illubabor	Yayu	Intermediate	Landrace
9	216737	West	Gambella	Zone 1	Itang	Lowland	Landrace
10	216743	West	Gambella	Zone 2	Abobo	Lowland	Landrace
11	223525	West	Oromiya	W.Wollega	Ghimbi	Lowland	Landrace
12	223543	West	Oromiya	Illubabor	Bure	Intermediate	Landrace
13	223548	West	Oromiya	Illubabor	Yayu	Lowland	Landrace
14	223551	West	Oromiya	Illubabor	Yayu	Lowland	Landrace
15	223552	West	Oromiya	Illubabor	Dedesa	Intermediate	Landrace
16	223554	West	Oromiya	Illubabor	Dedesa	Intermediate	Landrace
17	223555	West	Oromiya	Illubabor	Bedele	Intermediate	Landrace
18	223558	West	Oromiya	Jimma	Kerssa	Intermediate	Landrace
19	228736	Central	Oromiya	West Showa	Ambo	Lowland	Landrace
20	228739	Central	Oromiya	West Showa	Ambo	Lowland	Landrace
21	228740	Central	Oromiya	West Showa	Ambo	Lowland	Landrace
22	228741	Central	Oromiya	West Showa	Ambo	Lowland	Landrace
23	228919	West	Oromiya	Illubabor	Bure	Intermediate	Landrace
24	229831	Noth-west	BGRS	Metekel	Mandura	Lowland	Landrace
25	229834	Noth-west	BGRS	Metekel	Mandura	Lowland	Landrace
26	229835	Noth-west	BGRS	Metekel	Mandura	Lowland	Landrace
27	229838	Noth-west	BGRS	Metekel	Dibate	Lowland	Landrace
28	237762	Central	Oromiya	West Showa	BakoTibe	Intermediate	Landrace
29	237763	Central	Oromiya	West Showa	BakoTibe	Intermediate	Landrace
30	237779	West	Oromiya	West Wellega	Ghimbi	Lowland	Landrace
31	Geremw	Central	Oromiya	East Showa	Adama	Intermediate	Cultivar
32	97MW6129	Central	Oromiya	East Showa	Adama	Intermediate	Cultivar
33	97MW6127	Central	Oromiya	East Showa	Adama	Intermediate	Cultivar
34	NO253	N/a	ICRISAT	ICRISAT	N/a	Intermediate	Cultivar
35	PI308453	N/a	PU	PU	N/a	Intermediate	Cultivar
36	97MW6113	Central	Oromiya	East Showa	Adama	Intermediate	Cultivar
37	Macia-SA	North-west	North-west	North-west	Potchefstroom	Dry	Cultivar
38	M48	North-west	North-west	North-west	Potchefstroom	Dry	Cultivar
39	M141	North-west	North-west	North-west	Potchefstroom	Dry	Breeding line
40	M81	North-west	North-west	North-west	Potchefstroom	Dry	Breeding line
41	M105	North-west	North-west	North-west	Potchefstroom	Dry	Cultivar
42	M101	North-west	North-west	North-west	Potchefstroom	Dry	Breeding line
43	M163	North-west	North-west	North-west	Potchefstroom	Dry	Breeding line
44	Masekaswere	Limpopo	Limpopo	Limpopo	Sekhukhune	Dry	Landrace
45	Mamolokwane	Limpopo	Limpopo	Limpopo	Sekhukhune	Dry	Landrace
46	M153	North-west	North-west	North west	Potchefstroom	Dry	Cultivar

*Accession numbers/name as obtained from the Institute of Biodiversity, Ethiopia, and the ARC-GCI, South Africa; BGR=Benishangul-Gumuz Region; ICRISAT= International Crops Research Institute for the Semi-Arid Tropics; PU=Purdue University; N/a=Not available.

Table 2. *Eco*RI and *Mse*I adapter, primer+1 and primer+3 sequences used in AFLP analysis Restriction digestion and ligation.

Enzyme	Type	Sequence (5'-3')
*Eco*RI	Adapter-F	CTCGTAGACTGCGTACC
	Adapter-R	AATTGGACGCAGTCTAC
*Mse*I	Adapter-F	GACGATGAGTCCTGAG
	Adapter-R	TACTCAGGACTCAT
*Eco*RI	Primer+1	GACTGCGTACCAATTCA
	Primer+3	GACTGCGTACCAATTCACA
*Mse*I	Primer+1	GATGAGTCCTGAGTAAC
	Primer+3	GATGAGTCCTGAGTAACNN
		CNN=CAC, CAG, CTA, CTC, CTG, CTT

1x Promega*Taq* polymerase buffer, 2 mM $MgCl_2$, 200 μM of each dNTP, 100 μg ml^{-1} bovine serum albumin, 30 ng*Mse*I-primer+3, 30 ng*Eco*RI-primer+3 and 0.75 U Promega*Taq* DNA polymerase. The selective amplification cycling programme consisted of: one cycle of denaturation at 94°C for 5 min followed by one cycle of 30 s at 94°C, 30 s at 65°C and 60 s at 72°C. The annealing temperature was reduced by 1°C per cycle during the next eight cycles after which 25 cycles were performed at 94°C for 30 s, 56°C for 30 s and 72°C for 60 s followed by one last elongation of 5 min at 72°C. AFLP products were separated in denaturing polyacrylamide gels and DNA fragments were visualised using silver staining.

Gel electrophoresis

Polymerase chain reaction (PCR) products were mixed with 20 μl formamide dye [98% (v/v) de-ionized formamide; 10 mM EDTA, pH 8.0; 0.05% (w/v) bromophenol blue; 0.05% (w/v) xylene cyanol] and denatured by incubation for 5 min at 95°C. Mixtures were immediately placed on ice prior to loading. The PCR products (2.5 μl) were separated on 5% (w/v) denaturing polyacrylamide gels [19:1 acrylamide:bis-acrylamide; 7 M urea; 1x TBE buffer (89 mMTris-borate; 2.0 mM EDTA)].

Silver staining for DNA visualisation

The silver staining process for DNA visualisation of the denaturing acrylamide gels was done using the Silver Sequence™ DNA Sequencing System of Promega. Gels were fixed in 10% (v/v) acetic acid for 30 min and rinsed three times in de-ionized water, firstly for 10 min, followed by 5 min each the last two washes. Gels were stained in a solution of 0.1% (w/v) silver nitrate and 0.056% (v/v) formaldehyde for 30 min and rinsed in de-ionized water for 5 s before being immersed in a cold (4 to 10°C) developing solution [3% (w/v) sodium carbonate; 0.056% (v/v) formaldehyde and 0.002 mg ml^{-1}thiosulphate]. Gels were shaken manually in the developer until DNA fragments became visible. The 10% acetic acid was used to stop the developing process and shaking continued for a further 2 to 3 min. The gel was rinsed in de-ionised water and left upright to dry overnight at room temperature. A photograph of the gel was taken by exposing the photographic paper (Ilford multigrade IV RC de Luxe) directly under the gel to dim light for 20 s. This produced a negative image of the same size, as of the gel.

Data analysis

A binary matrix of specific AFLP fragments as present (1) or absent (0) was generated for each accession. Only reliable (between 300 and 700 bp) and repeatable bands (at least three repetitions) were considered. Pair-wise genetic distances were expressed as the complement of the Dice coefficient (Dice, 1945). Cluster analyses were performed using unweighted pair-group method using arithmetic averages (UPGMA) (Sokal and Michener, 1958). Statistical analyses were performed using NTSYS-pc version 2.21c (Exeter Software, NY, USA). Dendrograms were created using the Sequencial Agglomerative Hierarchical Nested (SAHN) programme of NTSYS and goodness of fit of clustering to data matrixes was calculated using COPH and MXCOMP programmes and correlated with the original distance matrices in order to test the association between the cluster in the dendrogram and the Dice matrix. Principal co-ordinate analysis (PCoA) employed the DCENTER and EIGEN procedures of NTSYS-pc.

AFLP data were evaluated using Shannon Weaver Diversity index (H') and polymorphic information content (PIC). Shannon Weaver Diversity index was calculated over all loci as described by Perry and Mclntosh (1991). The PIC for each primer combination was calculated, to know its capability of making distinctions, assess the quality of markers and to compare the effectiveness of each enzyme primer combination in detecting and providing genetic information (Lanteri et al., 2004). PIC was calculated according to the method of Riek et al. (2001) for the dominant marker as follows: $PIC = 1- [f^2 + (1- f)^2]$ where f is the frequency of the marker in the data set. PIC values were averaged to provide PIC value for a primer-pair. The level of polymorphism (%) was calculated as the ratio of the number of polymorphic amplified fragments to the total number of detected fragments. This formula as described by Shevchuk et al. (2009) is given as $P=n_p/(n_p+n_{np}) \times 100\%$, where n_p is the number of polymorphic PCR fragments and n_{np} is the non-polymorphic PCR fragments.

RESULTS AND DISCUSSION

Genetic information of AFLP markers

A total of 186 fragments were amplified using six AFLP primer combinations. The number of scorable fragments

Table 3. Genetic information generated by six AFLP primer combinations using 46 sorghum accessions.

Primer	TNF	NPF	MF	P (%)	PIC	H'
E-ACA/M-CAG	22	15	7	68.18	0.256	0.209
E-ACA/M-CTC	35	21	14	60.00	0.222	0.206
E-ACA/ M-CTT	36	12	24	33.33	0.139	0.203
E-ACA/ M-CTG	34	10	24	29.41	0.221	0.251
E-ACA/ M-CTA	29	9	20	31.03	0.123	0.146
E-ACA/M-CAC	30	11	19	36.67	0.106	0.123
Total	186	78	108			
Average	31	13	18	43.10	0.178	0.190

TNF, Total number of fragments; NPF, number of polymorphic fragments; MF, monomorphic fragments; P (%), percentage polymorphism; PIC, polymorphic information content; H', Shannon Weaver diversity index.

amplified by each AFLP primer combination varied from 22 for E-ACA/M-CAG to 36 for E-ACA/M-CTT with an average value of 31 per primer combination (Table 3). That was lower than the average value of 39.3 obtained by Uptmoor et al. (2003) using 28 AFLP primer combinations, but higher than the values reported by Iqbal et al. (2010) using 16 RAPD primer combinations.

A total of 78 polymorphic fragments were scored between the different sorghum accessions with the number of polymorphic fragments for each primer pair ranging from nine (31.03%) for E-ACA/M-CTA to 21 (60%) for E-ACA/M-CTC with an average value of 13 polymorphic fragments per primer combination (43.10%). A total of 108 (18%) monomorphic fragments were also detected. The E-ACA/M-CAG primer combination amplified the lowest bands (22) but at a higher rate of polymorphism (68.18%). The smaller number of fragments per primer combination detected in this study compared to previous studies may be due to the smaller number of primers. Ritter et al. (2007) reported an average value of 17 polymorphic fragments using 16 AFLP primer combinations on 95 sorghum lines that was higher than the values reported in this study. Furthermore, of the 598 scored fragments, 277 (46%) polymorphic fragments detected that was almost similar to the value obtained in the present study. Perumal et al. (2007) reported 30.53% polymorphic fragments using 16 AFLP primer combination using 46 converted sorghum lines which was lower than the value obtained in the present study. Uptmoor et al. (2003) also reported 61.80% polymorphic fragments among 46 South African sorghum accessions using AFLP markers and Geleta (2003) reported 85% using 45 sorghum accessions collected from the eastern parts of Ethiopia using eight primer combinations. Ayana et al. (2000) detected 69% polymorphic fragments among 93 individuals representing 11 wild sorghum populations in Ethiopia using RAPD. Iqbal et al. (2010) found 78.4% polymorphic fragments among 29 sorghum genotypes using RAPD markers in Pakistan and also they reported higher similarity among sorghum genotypes.

In the present study, primer combination E-ACA/M-CAG was highly discriminative compared to other enzyme primer combinations. The PIC and Shannon diversity index values for each primer combinations ranged from 0.106 to 0.256 with overall average of 0.178 and 0.123 to 0.251 with an overall mean of 0.190, respectively (Table 3). Primer enzyme combinations E-ACA/M-CAG revealed the highest PIC value which indicated its usefulness in differentiating individuals and presented high information content compared to other combinations. The number and frequency of the fragments affected the PIC values of the informativeness of the markers. The highest H' value of 0.251 was recorded for primer combination E-ACA/M-CTG. Primer combinations E-ACA/M-CAC showed the lowest values for PIC and H'. In the present study, the low levels of H' values indicated that there was low genetic diversity detected among the accessions tested. Genetic polymorphism present among sorghum accessions was low, as evidenced by the high level of similarity in the AFLP marker profiles of different sorghum germplasm accessions. The narrow genetic diversity detected in the present study may suggest sorghum breeding programmes of both Ethiopia and South Africa need new sources of genetic variation to bring the desired genetic improvement in sorghum.

Genetic distance similarity and cluster analysis

Estimates of genetic similarity matrices based on the AFLP marker data for all pair-wise combinations of the 46 sorghum accessions are presented in Table 4. The genetic similarity varied from 0.87 to 0.99. The high levels of genetic similarity indicated that accessions were related and the degree of variation was limited. Sampling more accessions from north-western, western and central parts of Ethiopia would be an effective way of capturing genetic variation for future collections.

The genetic similarity within the 30 landraces was found to be very high, with very few values lower than 0.90. Landrace 9 (Table 4) was the only one that was

Table 4. Genetic similarity among 46 sorghum accessions generated using six AFLP primer combinations based on Dice's similarity coefficient.

Number	Accession	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
1	69029	1.00																																												
2	69030	0.99	1.00																																												
3	69032	0.98	0.98	1.00																																											
4	69128	0.94	0.94	0.95	1.00																																									
5	69147	0.91	0.91	0.92	0.89	1.00																																								
6	69164	0.93	0.93	0.94	0.92	0.94	1.00																																							
7	69165	0.93	0.93	0.94	0.92	0.91	0.94	1.00																																						
8	69538	0.97	0.97	0.98	0.95	0.91	0.92	0.93	1.00																																					
9	216737	0.87	0.87	0.88	0.85	0.92	0.90	0.90	0.88	1.00																																				
10	216743	0.90	0.91	0.92	0.89	0.95	0.92	0.91	0.92	0.94	1.00																																			
11	223525	0.90	0.91	0.91	0.90	0.92	0.90	0.90	0.93	0.91	0.91	1.00																																		
12	223543	0.89	0.90	0.90	0.91	0.93	0.91	0.91	0.92	0.91	0.93	0.94	1.00																																	
13	223548	0.90	0.90	0.92	0.89	0.97	0.94	0.92	0.93	0.92	0.96	0.93	0.95	1.00																																
14	223551	0.93	0.92	0.93	0.90	0.87	0.89	0.89	0.94	0.90	0.87	0.94	0.89	0.89	1.00																															
15	223552	0.98	0.97	0.97	0.93	0.92	0.94	0.93	0.97	0.89	0.91	0.91	0.89	0.91	0.94	1.00																														
16	223554	0.97	0.96	0.96	0.93	0.91	0.93	0.92	0.96	0.88	0.90	0.91	0.89	0.91	0.94	0.99	1.00																													
17	223555	0.97	0.97	0.96	0.94	0.92	0.93	0.92	0.96	0.88	0.91	0.92	0.90	0.91	0.93	0.99	0.99	1.00																												
18	223558	0.93	0.93	0.93	0.92	0.93	0.93	0.93	0.95	0.92	0.92	0.94	0.93	0.94	0.91	0.95	0.94	0.94	1.00																											
19	228736	0.91	0.92	0.92	0.90	0.93	0.91	0.90	0.92	0.93	0.93	0.91	0.95	0.93	0.89	0.91	0.90	0.91	0.93	1.00																										
20	228739	0.97	0.96	0.96	0.93	0.90	0.92	0.92	0.97	0.87	0.89	0.93	0.91	0.91	0.96	0.97	0.97	0.96	0.94	0.91	1.00																								
21	228740	0.97	0.96	0.97	0.95	0.92	0.92	0.93	0.98	0.90	0.91	0.93	0.93	0.93	0.98	0.97	0.98	0.98	0.95	0.92	0.99	1.00																							
22	228741	0.96	0.94	0.95	0.92	0.90	0.91	0.91	0.97	0.88	0.90	0.92	0.92	0.92	0.97	0.96	0.96	0.95	0.93	0.91	0.99	0.99	1.00																						
23	228919	0.96	0.95	0.95	0.93	0.90	0.91	0.91	0.96	0.86	0.89	0.92	0.91	0.91	0.95	0.96	0.96	0.96	0.93	0.92	0.98	0.98	0.97	1.00																					
24	229831	0.89	0.89	0.90	0.88	0.94	0.92	0.91	0.90	0.92	0.94	0.90	0.95	0.95	0.87	0.89	0.89	0.90	0.92	0.93	0.89	0.92	0.89	0.89	1.00																				
25	229834	0.95	0.93	0.94	0.92	0.89	0.91	0.93	0.93	0.88	0.88	0.92	0.90	0.89	0.93	0.93	0.93	0.93	0.92	0.91	0.95	0.97	0.94	0.95	0.89	1.00																			
26	229835	0.95	0.94	0.94	0.92	0.92	0.93	0.94	0.95	0.88	0.91	0.90	0.92	0.93	0.91	0.94	0.94	0.94	0.93	0.93	0.95	0.95	0.94	0.94	0.92	0.95	1.00																			
27	229838	0.92	0.92	0.92	0.92	0.94	0.96	0.94	0.93	0.90	0.92	0.91	0.92	0.93	0.88	0.93	0.92	0.93	0.94	0.92	0.91	0.93	0.91	0.91	0.92	0.92	0.94	1.00																	
28	237762	0.88	0.89	0.89	0.87	0.93	0.89	0.90	0.91	0.92	0.92	0.93	0.94	0.95	0.88	0.89	0.89	0.90	0.94	0.93	0.91	0.92	0.91	0.91	0.94	0.91	0.90	0.92	1.00															
29	237763	0.94	0.93	0.94	0.90	0.88	0.89	0.89	0.96	0.87	0.88	0.92	0.91	0.89	0.97	0.94	0.94	0.93	0.91	0.90	0.97	0.99	0.98	0.95	0.89	0.94	0.92	0.89	0.91	1.00														
30	237779	0.88	0.90	0.90	0.88	0.95	0.92	0.91	0.91	0.91	0.95	0.94	0.94	0.96	0.88	0.90	0.90	0.91	0.94	0.92	0.90	0.91	0.90	0.90	0.94	0.89	0.91	0.92	0.95	0.89	1.00													
31	Geremw	0.91	0.91	0.91	0.90	0.92	0.93	0.92	0.93	0.90	0.93	0.90	0.90	0.93	0.88	0.91	0.91	0.93	0.91	0.89	0.90	0.92	0.89	0.90	0.92	0.90	0.92	0.92	0.89	0.88	0.93	1.00												
32	97MW6129	0.89	0.88	0.89	0.88	0.90	0.89	0.91	0.90	0.91	0.92	0.89	0.92	0.91	0.90	0.89	0.89	0.89	0.90	0.92	0.90	0.93	0.90	0.91	0.92	0.93	0.92	0.90	0.92	0.90	0.90	0.91	1.00											
33	97MW6127	0.89	0.88	0.89	0.88	0.89	0.88	0.92	0.90	0.91	0.90	0.90	0.90	0.90	0.88	0.89	0.89	0.89	0.89	0.91	0.89	0.92	0.89	0.91	0.90	0.93	0.90	0.90	0.92	0.92	0.88	0.90	0.98	1.00										
34	NO253	0.89	0.88	0.88	0.86	0.87	0.88	0.89	0.90	0.89	0.89	0.89	0.90	0.88	0.88	0.89	0.89	0.89	0.88	0.91	0.88	0.91	0.89	0.90	0.91	0.92	0.90	0.89	0.90	0.91	0.87	0.91	0.96	0.95	1.00									
35	PI308453	0.91	0.91	0.90	0.89	0.92	0.92	0.92	0.91	0.93	0.94	0.90	0.91	0.92	0.89	0.92	0.92	0.92	0.92	0.92	0.90	0.93	0.89	0.91	0.92	0.92	0.93	0.92	0.91	0.89	0.92	0.94	0.97	0.96	0.94	1.00								
36	97MW6113	0.92	0.92	0.92	0.89	0.92	0.93	0.92	0.92	0.93	0.94	0.91	0.92	0.92	0.89	0.93	0.93	0.93	0.94	0.92	0.91	0.93	0.90	0.90	0.94	0.91	0.93	0.93	0.90	0.90	0.93	0.94	0.94	0.93	0.95	0.97	1.00							
37	Macia SA	0.91	0.91	0.92	0.89	0.94	0.92	0.91	0.92	0.95	0.97	0.89	0.93	0.94	0.88	0.92	0.92	0.93	0.93	0.94	0.90	0.93	0.90	0.89	0.95	0.89	0.92	0.92	0.91	0.89	0.94	0.93	0.93	0.92	0.91	0.95	0.96	1.00						
38	M48	0.89	0.91	0.90	0.88	0.91	0.91	0.92	0.92	0.91	0.91	0.91	0.91	0.90	0.88	0.91	0.91	0.91	0.92	0.90	0.90	0.93	0.89	0.90	0.93	0.91	0.91	0.92	0.91	0.89	0.91	0.93	0.93	0.93	0.92	0.95	0.96	0.93	1.00					
39	M141	0.89	0.89	0.90	0.87	0.90	0.90	0.90	0.91	0.92	0.92	0.88	0.92	0.91	0.88	0.90	0.89	0.90	0.90	0.92	0.88	0.91	0.88	0.89	0.92	0.90	0.90	0.90	0.91	0.89	0.90	0.91	0.95	0.93	0.93	0.94	0.94	0.92	0.92	1.00				
40	M81	0.89	0.89	0.90	0.88	0.93	0.91	0.91	0.90	0.91	0.92	0.88	0.92	0.92	0.87	0.90	0.89	0.90	0.91	0.91	0.89	0.91	0.88	0.89	0.93	0.90	0.91	0.90	0.91	0.88	0.92	0.91	0.93	0.92	0.90	0.93	0.92	0.95	0.92	0.95	1.00			
41	M105	0.88	0.87	0.89	0.87	0.91	0.90	0.90	0.90	0.93	0.93	0.88	0.93	0.92	0.88	0.89	0.88	0.88	0.90	0.91	0.89	0.91	0.88	0.88	0.92	0.89	0.90	0.89	0.90	0.89	0.91	0.90	0.93	0.92	0.90	0.93	0.93	0.94	0.91	0.96	0.97	1.00		
42	M101	0.89	0.88	0.90	0.87	0.88	0.88	0.87	0.92	0.91	0.89	0.88	0.92	0.89	0.91	0.90	0.89	0.89	0.89	0.94	0.91	0.94	0.92	0.89	0.90	0.89	0.90	0.87	0.88	0.93	0.88	0.87	0.90	0.88	0.90	0.90	0.91	0.92	0.89	0.92	0.91	0.93	1.00	
43	M163	0.91	0.91	0.90	0.89	0.89	0.90	0.92	0.93	0.91	0.91	0.89	0.91	0.89	0.90	0.92	0.91	0.93	0.92	0.92	0.91	0.94	0.92	0.92	0.92	0.91	0.92	0.91	0.91	0.92	0.89	0.91	0.93	0.93	0.92	0.94	0.94	0.94	0.94	0.93	0.92	0.92	0.93	1.00
44	Masekaswere	0.88	0.88	0.88	0.86	0.88	0.90	0.90	0.90	0.91	0.90	0.89	0.91	0.89	0.88	0.89	0.89	0.89	0.91	0.91	0.88	0.91	0.88	0.88	0.91	0.90	0.89	0.90	0.90	0.90	0.90	0.91	0.93	0.92	0.94	0.93	0.94	0.91	0.92	0.95	0.92	0.92	0.93	0.93	1.00	
45	Mamolokwane	0.91	0.90	0.91	0.89	0.92	0.92	0.90	0.92	0.89	0.93	0.91	0.90	0.92	0.88	0.91	0.91	0.91	0.91	0.91	0.90	0.92	0.90	0.92	0.91	0.92	0.91	0.90	0.90	0.89	0.92	0.94	0.94	0.93	0.93	0.95	0.94	0.93	0.92	0.94	0.93	0.92	0.91	0.92	0.93	1.00
46	M153	0.89	0.88	0.90	0.89	0.90	0.89	0.90	0.91	0.91	0.92	0.88	0.92	0.91	0.89	0.90	0.90	0.90	0.90	0.92	0.89	0.91	0.90	0.90	0.90	0.89	0.90	0.89	0.90	0.89	0.90	0.90	0.94	0.93	0.92	0.94	0.92	0.94	0.91	0.94	0.94	0.95	0.93	0.93	0.92	0.94

less similar (<0.90) in comparison to nine other landraces, but the values were still higher than 0.80. This indicates that the landraces were genetically very similar, and were related to each other. Farmers may have exchanged seeds in the collection areas and crosses could have been made within the material, by cross pollination. However, AFLP analysis detected no duplications (100% similarity) within the tested accessions. There are some landraces that showed relatively low genetic similarity (<0.90) in pairwise comparisons with the Ethiopian sorghum cultivars as well as South African breeding lines, showing more dissimilarity within landraces than cultivars and breeding lines. Similarly, Kumar and Kumar (2009) found 100 genetic similarity coefficients among 40 sorghum genotypes using 11 SSR markers. Similarity within the Ethiopian cultivars and within the South African breeding material was very high, highlighting the importance of evaluating large numbers of accessions when searching for valuable traits for both breeding programmes.

Menz et al. (2004) reported genetic similarity ranging from 0.81 to 0.91 for 50 sorghum inbred lines using AFLP marker analysis. The average genetic similarity coefficient for the pair-wise combinations was 0.92. Uptmoor et al. (2003) reported mean genetic similarities among 46 sorghum accessions of 0.88 based on AFLP analysis. Similarly, Geleta et al. (2006) reported a genetic distance coefficient of 0.62 among 45 sorghum accessions using eight AFLP primer combinations. Furthermore, Abu Assar et al. (2005) found an average genetic similarity value of 0.30 among 96 sorghum genotypes which was much lower than the values reported in this study.

The dendrogram produced four distinct clusters (Figure 1). Cluster I comprised of 13 accessions at a genetic similarity of 0.932. All accessions in this cluster were cultivars except accessions Mamolokwane and Masekaswere. These were the two landrace accessions from South Africa and were separated from the subgroups as singletons.

In this main cluster (I), accessions were grouped mainly according to geographical origin. All accessions from South Africa, except Macia-SA and M101 clustered together in Cluster I. Clustering of the Ethiopian accessions with the South African ones might be due to the same reasons as hypothesised before, that they have a common source of ancestry. The only accessions that did not cluster in cluster I were Geremw, Macia-SA and M101. Accessions such as 97MW6129, 97MW6127 and 97MW6113 were advanced lines from Ethiopia, and NO253 and PI308453 were introductions from ICRISAT and Purdue University. The rest of the accessions in this cluster were breeding lines from South Africa that had undergone higher levels of selection. Selection thus might have narrowed down genetic diversity in the breeding lines, thus clustering together despite their geographical origin. The two exotic germplasm accessions, NO253 and PI308453 showed the degree of diversity between them and with accessions from Ethiopia and South Africa. Moreover, AFLP markers detected that they were distinctly related in the cluster though separated into different sub-groups within a cluster. Therefore, characterisation and identification of intra-specific accessions is a crucial factor for the success of introgression breeding in sorghum breeding programmes. Similarly, Uptmoor et al. (2003) detected genetic relatedness and diversity within 46 sorghum accessions grown in Southern Africa that revealed a clear separation between landraces and breeding cultivars using AFLP, RAPD and SSR markers. The clustering of accessions based on their genetic similarity in this study would help in selection of genetically diverse parental lines to get superior recombinants for future sorghum breeding programmes (Jeya et al., 2006).

Similarly, cluster II consisted of 13 accessions at a genetic distance of 0.912. This cluster was subdivided into two subgroups at a genetic similarity of 0.914 and contained accessions M101 (South Africa) and 228736 (Ethiopia) in one of the subgroups. Accessions M101 and Macia-SA were from South Africa and clustered together in cluster II along with 11 landrace accessions of Ethiopia. This must be due to some genetic relationships with the rest of the accessions in the cluster. All 11 landrace accessions in cluster II were collected from the north-western and western agro-ecologies of Ethiopia, although they were from different localities (Table 1). Accessions 237762 and 228736 were collected from central Ethiopia in the west Showa zone and grouped in this cluster based on regional backgrounds. This might be due to gene flow, and seed exchange among farmers within and between regions, thus causing clustering. Bucheyeki et al. (2009) reported the clustering of 40 sorghum landraces using SSR-based analysis in Tanzania based on their area of collection sites, pedigree relationship, variations and diversity of the landraces. According to Barnaud et al. (2008), Bucheyeki et al. (2009) and Muray et al. (2010), the gene flow plays a large role in structuring the genetic variability within and among sorghum populations. Manzelli et al. (2007) reported continuous exchanges of genes between sorghum population results in genetic diversity.

Cluster III comprised of four accessions at a genetic distance similarity of 0.926. This group contained landrace accessions from the north-western parts of Ethiopia that were collected from the Metekel zone of the Benishangul-Gumuz region, except for cultivar Geremw, which was obtained from Melkassa Agricultural Research Center (MARC) and was an outlier in this cluster.

Cluster IV contained 16 accessions which was a mixture of accessions collected from the north-western and western as well as central parts of Ethiopia. Accession 69128 clustered separately from the other accessions in the cluster and was collected from the Assosa zone of the Benishangul-Gumuz region, border

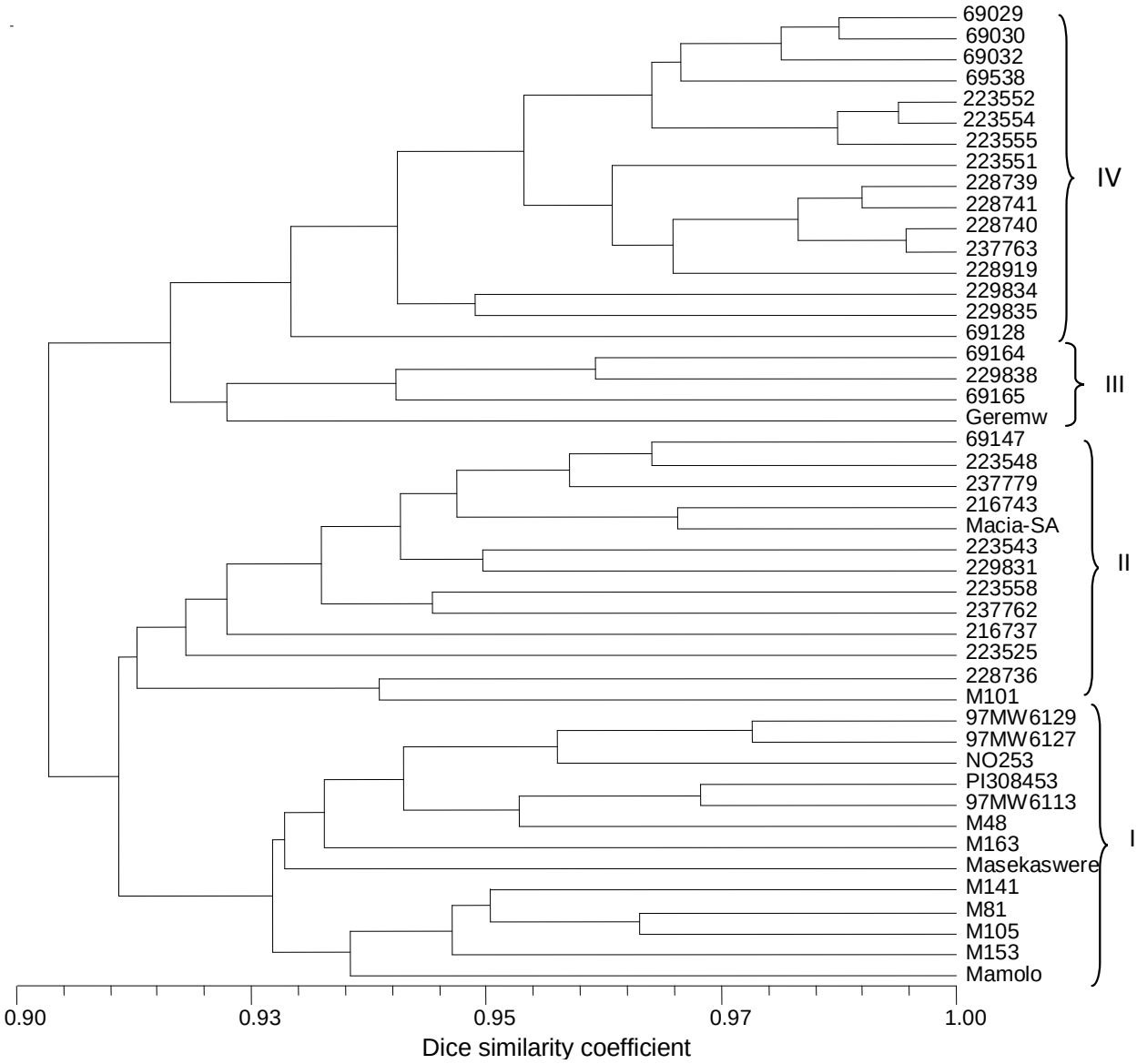

Figure 1. Dendrogram revealing genetic relationships among 46 sorghum accessions from Ethiopia and South Africa based on AFLP analysis, Dice similarity coefficients and UPGMA clustering, Mamolo=Mamolokwane.

to South Sudan.

Accessions Geremw, 223525, Masekaswere, Mamolokwane, M163, M153 and 69128 clustered separately from the rest of the accessions in their group, but that only made them genetically distinct in that particular group, not with all accessions tested. The presence of this difference within the cluster will have benefits in breeding programmes and selection of parental lines. Similarly, Mohanraj et al. (2006) reported that the maximum amount of heterosis is expected from the crosses with parents belonging to the most divergent Clusters.

Accessions 228740 and 237763 were the most similar of all accessions evaluated. Both these accessions were from the west Showa zones of the Oromia region (with a similarity coefficient of 0.994). The two collection sites, Ambo and BakoTibe, were close in distance to each other in the west Showa zone. Similarly, accessions 223552 and 223554 were the second most similar accessions, both collected from Dedesa in the Illubabor zone.

Accessions 228739, 228741, 228740 and 237763 that clustered closely together in cluster IV, were collected from the Ambo and BakoTibe districts of the west Showa zone in the central parts of Ethiopia. These accessions clustered closely together with accessions 223551 and 228919 that were collected from west of Ethiopia from the Illubabor zone (Yayu and Bure Districts). Many accessions

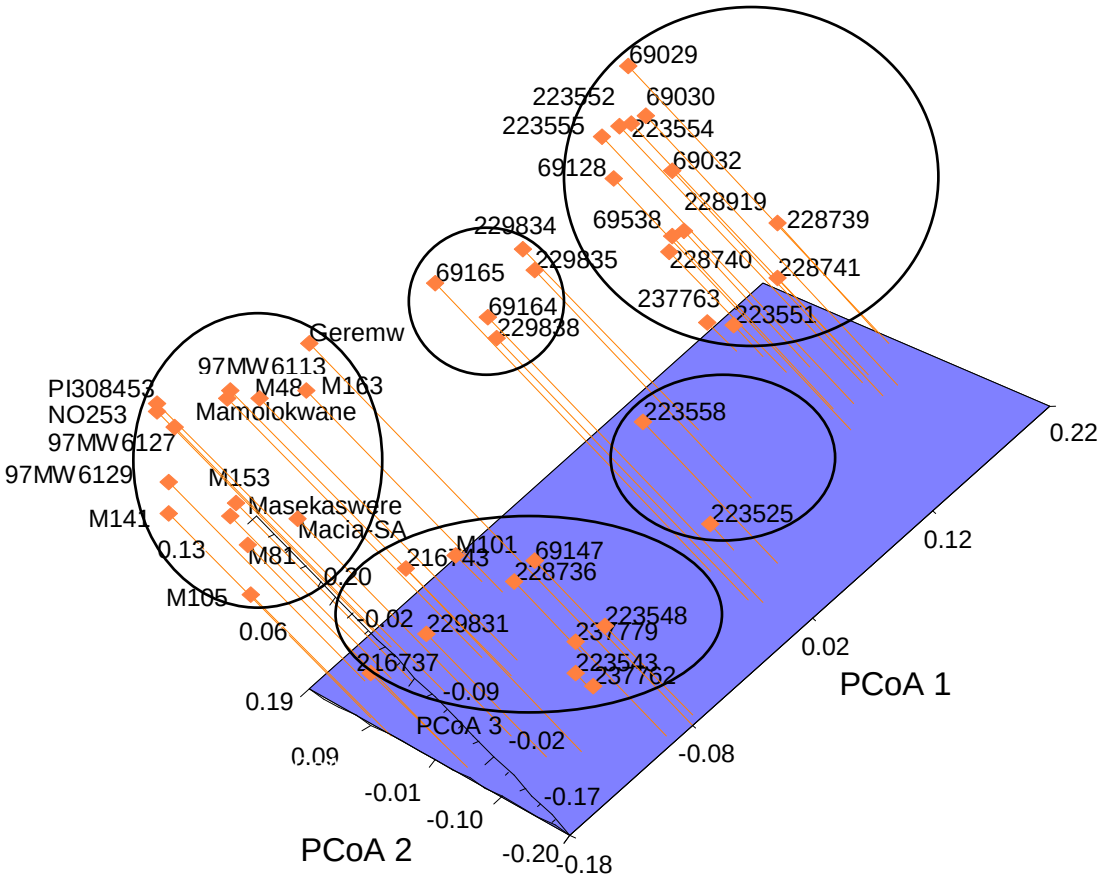

Figure 2. Principal co-ordinate analysis biplot for genetic characterisation of 46 sorghum accessions using AFLP analysis.

from the same region of origin and those closely situated regions clustered together. Regions in close geographical proximities, example Benishangul-Gumuz Region and Oromia regions, clustered together (cluster IV). Similarly, Vittal et al. (2010) reported that SSR markers grouped 23 sorghum genotypes from the United State of America based on their geographical origin. Moreover, Shehzad et al. (2009) also found that 320 sorghum accessions from Ukraine were distinctly classified according to geographic distribution. In line with this, Abu Assar et al. (2005) reported that 96 sorghum genotypes from Sudan grouped together based on their geographical origin as well as adaptation zones. Folkertsma et al. (2005) also reported on the variation among Guinea-race sorghum landraces based on eco-geographical regions using SSR markers.

Principal coordinate analysis using AFLP markers

The PCoA biplot (Figure 2) clustered accessions similarly to the dendrogram based on their genetic similarity with some differences. Accessions grouping together in cluster I of the dendrogram (Figure 1) were also grouped together in the biplot. Similarly, those accessions grouping together in clusters II, III, and IV also grouped together in the biplot with some exceptions. Accessions Geremw, 69165, 229838 and 69164 clustered together in the dendrogram, but in the biplot cultivar Geremw was separated from the group and clustered together with other commercial cultivars. This might be due to the pedigree relationships with the rest of the accessions in cluster I of the dendrogram. Accessions 229834 and 229835 clustered together with accessions 69164, 69165 and 229838, all collected from the Metekel zone in Ethiopia.

Accessions Macia-SA, 223525 and 223558 clustered together in cluster II of the dendrogram (Figure 1) but with the PCoA, they appeared to be far apart from the group and fell in different groups. Macia-SA grouped together with the cultivars, whereas, accessions 223525 and 223558, (both) collected from the Ghimbi and Keresa districts of the Oromia region clustered together and both these districts were in close proximity to each other. Therefore, the PCoA separated accessions better than the dendrogram based on the genetic similarity analysis and their geographical location.

Conclusions

Understanding the genetic diversity of sorghum germplasm collections is important for effective and efficient exploitation of their genetic potential as well as for selection of landraces and other genotypes as breeding material, maintenance and for conservation. The AFLP marker analysis has provided low level of genetic diversity within the tested sorghum accessions. One reason for the low levels of genetic diversity among Ethiopian landraces might be due to the small number of samples included and the factor being that a few number of AFLP primers used.

AFLP marker data identified and clustered accessions mainly according to their collection sites. The PCoA provided a similar structure but with some differences to that of the dendrogram's clustering patterns, suggesting the effectiveness and efficiency of PCoA analysis in genetic diversity analysis. Based on this finding, we recommend further investigation using more AFLP primers and larger number of accessions from wider sorghum growing areas of the country.

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