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Short communication

Analysis of genetic variation in sorghum (*Sorghum bicolor* (L.) Moench) genotypes with various agronomical traits using SPAR methods

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ABSTRACT

Genetic variation among 45 genotypes of sorghum (*Sorghum bicolor* L.) representing seven subpopulations was assessed using three single primer amplification reaction (SPAR) methods viz., inter-simple sequence repeat (ISSR), random amplified polymorphic DNA (RAPD) and directed amplification of minisatellite-region DNA (DAMD). Totally 15 ISSR, 8 RAPD and 7 DAMD primers generated 263 amplification products, accounting for 84.6% polymorphism across all the genotypes. The Mantel's test of correlation revealed the best correlation between ISSR and cumulative data with a correlation coefficient (r) of 0.84. Assessment of population diversity indicated that the maximum intra population genetic diversity was recorded among high FeZn lines (HFL) having maximum values of Nei's genetic diversity (h) (0.244), Shannon information index (I) (0.368) and the percentage of polymorphic loci (Pp) (72.65%) while the corresponding lowest values of 0.074, 0.109 and 17.95% respectively were observed among the members of MDT subpopulation. The mean coefficient of gene differentiation (GST) and the gene flow (Nm) between populations were observed to be 0.396 and 0.7680 respectively. The analysis of molecular variance (AMOVA) suggested that maximum genetic variation exists within populations (95%) than among populations (5%). Thus the information obtained from this study could be utilized in sorghum breeding programmes for the development of varieties with improved nutrition and agronomic values in future.

1. Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) ranked five in the list of world's important cereal crops and it plays a unique role in food security and renewable source (Belton and Taylor, 2004). It is the chief staple food, feed and fodder and cultivated globally with an annual average production of 61 million tonnes (FAO, 2004). The global area engaged in sorghum cultivation is 46 Mha (FAO, 2009). Due to an excellent adaptation to drought, water logging and salinity, sorghum has become a crop of choice for unimproved soils where growth of other cereals such as maize cannot be possible (ICRISAT, 1996). Thus the genetic improvement of sorghum for tolerance to drought and salinity through breeding would help to further increase its productivity in soils prone to drought and salinity. Micronutrient deficiency is the major global health problem and more than 2 billion people in the world are estimated to be deficient in key vitamins and minerals, particularly vitamin A, iodine, iron and zinc (FAO, 2011). Next to pearl millet, sorghum is the second cheapest source of energy and micronutrients and a huge mass of the population in Africa and central India depend on sorghum for their dietary energy and micro-nutrient requirements (Rao, 2006). Therefore biofortification of sorghum with improved micronutrients (especially iron and zinc) is also one of the widespread interest of the researchers (Pfeiffer and McClafferty, 2007).

Analyses of the extent and distribution of genetic variations between and within populations in a crop are essential in understanding the evolutionary relationships and to sample genetic resources in a more organized fashion for breeding and conservation purposes (Milligan et al., 1994). Different types of molecular markers have been successfully employed for the purposes of linkage mapping and diversity analysis in sorghum such as random amplified polymorphic

Abbreviations: AMOVA, analysis of molecular variance; DAMD, directed amplification of minisatellite-region DNA; FAO, Food and Agriculture Organization; HFL, high Fe–Zn lines; ISSR, inter-simple sequence repeat; MDS, mid-season drought susceptible; MDT, mid-season drought tolerant; OG, out group; PCR, polymerase chain reaction; PIC, polymorphic information content; RAPD, random amplified polymorphic DNA; Rp, resolving power; SG, stay green; SPAR, single primer amplification reaction; SS, saline susceptible; ST, saline tolerant; TDT, terminal drought tolerant; UPGMA, unweighted pair group method arithmetic average.

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DNA (RAPD) (Akram et al., 2011; Dahlberg et al., 2002), simple sequence repeat (SSR) (Kong et al., 2000; Wang et al., 2013), inter-simple sequence repeat (ISSR) (Medraoui et al., 2007), restriction fragment length polymorphism (RFLP) (Chantereau et al., 2001, Deu et al., 1994), amplified fragment length polymorphism (AFLP) (Zhu-Salzman et al., 2003) and diversity array technology (DArT) (Mace et al., 2009). However, studies focusing on identification of genetic relationships among genotypes with various agronomic traits such as improved nutritional values and susceptibility/tolerance to biotic and abiotic stresses are limited in sorghum. Recently, molecular markers based on SPAR (single primer amplification reaction) approaches are gaining importance as effective tools for genetic diversity analysis in higher plants which mainly include the random amplified polymorphic DNA (RAPD) (Williams et al., 1990), inter-simple sequence repeat (ISSR) (Gupta et al., 1994) and directed amplification of minisatellite-region DNA (DAMD) (Heath et al., 1993). Thus the present study was undertaken to investigate the extent of genetic variation among sorghum subpopulations of various agronomical traits (tolerant and sensitive to drought and saline and with high Fe–Zn content) using SPAR methods viz., ISSR, RAPD and DAMD markers so as to provide a baseline data for sorghum breeders for biofortification and development of abiotic stress tolerant sorghum varieties in future.

Table 1
Details of 45 genotypes of sorghum used for SPAR marker analysis in the present study.

S. no.	Accession	Agronomic trait of accession	Group label
1.	ICSV 210	Mid-season drought tolerant	MDT
2.	S 35	Mid-season drought tolerant	MDT
3.	ICSV 112	Mid-season drought tolerant	MDT
4.	ICSV 197	Mid-season drought susceptible	MDS
5.	ICSV 745	Mid-season drought susceptible	MDS
6.	ICSV 1	Mid-season drought susceptible	MDS
7.	ICSA 675	Stay green lines	SG
8.	ICSA 676	Stay green lines	SG
9.	ICSA 677	Stay green lines	SG
10.	ICSA 678	Stay green lines	SG
11.	ICSB 351	Stay green lines	SG
12.	ICSB 675	Stay green lines	SG
13.	ICSB 676	Stay green lines	SG
14.	ICSB 677	Stay green lines	SG
15.	ICSB 678	Stay green lines	SG
16.	M 35	Terminal drought tolerant	TDT
17.	ICSA 25	Terminal drought tolerant	TDT
18.	ICSB 25	Terminal drought tolerant	TDT
19.	ICSR 143	Saline tolerant	ST
20.	ICSB 143	Saline tolerant	ST
21.	ICSR 89012	Saline tolerant	ST
22.	ICSR 93011	Saline tolerant	ST
23.	ICSR 93024	Saline tolerant	ST
24.	ICSA 102	Saline tolerant	ST
25.	ICSB 102	Saline tolerant	ST
26.	ICSA 338	Saline susceptible	SS
27.	ICSB 338	Saline susceptible	SS
28.	ICSV 93042	Saline susceptible	SS
29.	ICSV 95126	Saline susceptible	SS
30.	ICSA 73	Saline susceptible	SS
31.	ICSB 73	Saline susceptible	SS
32.	ICSA 17	High Fe–Zn lines	HFL
33.	ICSA 263	High Fe–Zn lines	HFL
34.	ICSA 344	High Fe–Zn lines	HFL
35.	ICSA 351	High Fe–Zn lines	HFL
36.	ICSA 399	High Fe–Zn lines	HFL
37.	ICSB 17	High Fe–Zn lines	HFL
38.	ICSB 263	High Fe–Zn lines	HFL
39.	ICSB 344	High Fe–Zn lines	HFL
40.	ICSB 399	High Fe–Zn lines	HFL
41.	ICSR 89035	High Fe–Zn lines	HFL
42.	ICSR89035A	High Fe–Zn lines	HFL
43.	ICSR 40	High Fe–Zn lines	HFL
44.	ICSR 113	High Fe–Zn lines	HFL
45.	WILD	Out group	OG

2. Materials and methods

2.1. Plant materials and genomic DNA isolation

A random minicore collection of 44 genotypes of sorghum representing the seven subpopulations viz., mid-season drought tolerant (MDT), mid-season drought susceptible (MDS), stay green (SG), terminal drought tolerant (TDT), saline tolerant (ST), saline susceptible (SS) and high Fe–Zn containing (HFL) lines were obtained from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India. A wild genotype was procured from Tamil Nadu Agricultural University (TNAU), Coimbatore, India and used as an out group for this study. The details of these genotypes are given in Table 1. All 45 genotypes were grown in a shade house. Fresh leaf samples from 1 month old plants were used for genomic DNA extraction using CTAB method (Doyle and Doyle, 1990). The quality of genomic DNA was checked by 0.8% agarose gel electrophoresis and quantified using a UV–Visible Spectrophotometer (BioSpec Nano, Shimadzu, Japan).

2.2. ISSR, RAPD and DAMD analysis

A set of 45 anchored microsatellite primers, 30 random decamers and 25 minisatellite primers (Sigma, USA) were surveyed preliminarily, among which 15 ISSR, 8 RAPD and 7 DAMD primers produced well distinguished bands on polyacrylamide gel and these were selected for the amplification of 45 genotypes. The details of these primers are given in Table 2. The PCR cocktail and thermal cycling conditions were same for all three SPAR methods except the annealing temperatures. PCR reactions were set out in 12.5 μl volumes containing 30 ng of template DNA, 1× *Taq* buffer, 1.5 mM $MgCl_2$, 200 μM each of dNTPs, 0.4 μM primers and 1 unit of *Taq* DNA polymerase (MBI, Fermentas, Germany). The amplification was performed in a thermal cycler (AB Applied Biosystems, USA) with the following conditions: initial denaturation at 94 °C for 5 min, 42 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C (for ISSR and DAMD analysis) and 37 °C (for RAPD analysis) for 1 min each, extension at 72 °C for 2 min and final extension at 72 °C for 7 min. The amplified PCR products were electrophoresed on 8% polyacrylamide gel using 0.5× TBE buffer at a constant voltage of 5 V/cm. The gel was visualized by ethidium bromide staining and archived using gel documentation system (Molecular Imager® Gel Doc™ XR^+ System, Bio-Rad, USA). The patterns were photographed and stored as digital pictures in gel documentation system for future analyses.

2.3. Scoring and data analyses

The binary data were scored as the presence (1) or absence (0) of a band. The capability of each marker to distinguish between genotypes was analyzed by calculating the Resolving Power (Rp) using the formula $Rp = \Sigma Ib$ where Ib is the band informativeness; $Ib = 1 - (2 \times (0.5 - p))$ and p is the proportion of genotypes containing band I (Prevost and Wilkinson, 1999). The polymorphic information content (PIC) was calculated according to Botstein et al. (1980). A pair wise matrix of similarity between accessions was determined using Jaccard's similarity coefficient of the NTSYS-pc version 2.1 software (Rohlf, 1998). To obtain the genetic relationships among 45 genotypes, the genetic similarity matrix was constructed into a dendrogram using the UPGMA method. The genetic diversity parameters such as Nei's gene diversity (h), Shannon's information index (I) and the percentage of polymorphic loci (Pp) were estimated using POPGENE program v. 1.31 (Yeh et al., 1999). Gene flow (Nm) between populations was estimated from $Nm = 0.5\ (1 - GST)/GST$. The analysis of molecular variance (AMOVA) was carried out using the program GenAlEx v. 6.5 (Peakall and Smouse, 2012). The Mantel Z-statistic was used to test

Table 2
Details on polymorphism obtained by ISSR, RAPD and DAMD markers with 45 genotypes of sorghum.

Sl. no	SPAR method	Primer sequence (5′–3′)	Size range of amplicons (bp)	TNB	NMB	NPB	PPM (%)	R_p	PIC
ISSR									
1	ISSR A7	AGGAGGAGGAGGAGGAGG	250–1000	4	1	3	75	2.68	0.66
2	ISSR A8	ACACACACACACACACT	250–1500	5	0	5	100	6.79	0.88
3	ISSR A10	AGAGAGAGAGAGAGAGG	200–2500	9	1	8	88.89	10.7	0.63
4	ISSR A11	GAGAGAGAGAGAGAGAGAT	500–2500	4	0	4	100	4.48	0.57
5	ISSR Y5	CTCTCTCTCTCTCTCTAC	200–1500	8	1	7	87.5	5.11	0.85
6	ISSR Y11	GAGAGAGAGAGAGAGAT	250–1000	8	2	6	75	6.91	0.86
7	ISSR 33	AGAGAGAGAGAGAGAGA	200–1500	7	2	5	71.43	6.89	0.83
8	ISSR 35	AGAGAGAGAGAGAGAGT	400–1750	8	1	7	87.5	3.3	0.80
9	ISSR 42	ACACACACACACACACCG	300–1500	9	1	8	88.89	5.91	0.89
10	ISSR 46	ACACACACACACACACGG	200–1750	19	2	17	89.47	11.45	0.64
11	ISSR 67	TCTCTCTCTCTCTCCC	300–1800	7	1	6	85.71	4.84	0.90
12	HB 9	GTGTGTGTGTGTGG	180–3000	5	1	4	80	6.04	0.81
13	HB 15	GTTGTTGTTGC	100–3000	15	1	14	92.86	10.87	0.81
14	17898B	CACACACACACAGT	100–2000	15	1	14	92.86	6.88	0.86
15	HB12	CACCACCACGC	280–3500	9	1	8	88.89	3.07	0.70
		Average		132	16	116	86.93	6.39	0.78
RAPD									
1	OPH3	AGACGTCCAC	250–1000	4	1	3	75	8.34	0.84
2	OPG2	GGCACTGAGG	250–1500	5	0	5	100	7.06	0.90
3	OPD10	GGTCTACACC	200–2500	9	1	8	88.89	6.74	0.86
4	OPD8	GTGTGCCCCA	500–2500	4	0	4	100	11.1	0.71
5	OPD18	GAGAGCCAAC	280–3500	9	1	8	88.89	7.22	0.81
6	OPD13	GGGGTGACGA	250–3000	8	0	8	100	5.51	0.85
7	OPA15	TTCCGAACCC	300–2000	4	0	4	100	3.16	0.73
8	OPC19	GTTGCCAGCC	400–3500	6	0	6	100	4.92	0.89
		Average		49	3	46	94.09	6.75	0.82
DAMD									
1	URP2R	CCCAGCAACTGATCGCACAC	90–8000	20	7	13	65	11.62	0.19
2	URP4R	AGGACTCGATAACAGGCTCC	400–8500	11	3	8	72.7	6.93	0.67
3	URP9F	ATGTGTGCGATCAGTTGCTG	400–2500	14	3	11	78.57	4.55	0.77
4	URP25F	GATGTGTTCTTGGAGCCTGT	750–3500	7	4	3	42.35	10.85	0.77
5	URP30F	GGACAAGAAGAGGATGTGA	150–3500	14	3	11	78.57	9.97	0.50
6	URP38F	AAGAGGCATTCTACCACCAC	200–1500	9	0	9	100	7.77	0.75
7	M13	GAGGGTGGCGGCTCT	300–2000	7	2	5	72.43	6.82	0.69
		Average		82	22	60	72.80	8.35	0.62
Cumulative (30 primers)				263	41	222	84.60	7.16	0.74

TNB, total number of bands; NMB, number of monomorphic bands; NPB, number of polymorphic bands; Rp, resolving power; PIC, polymorphic information content.

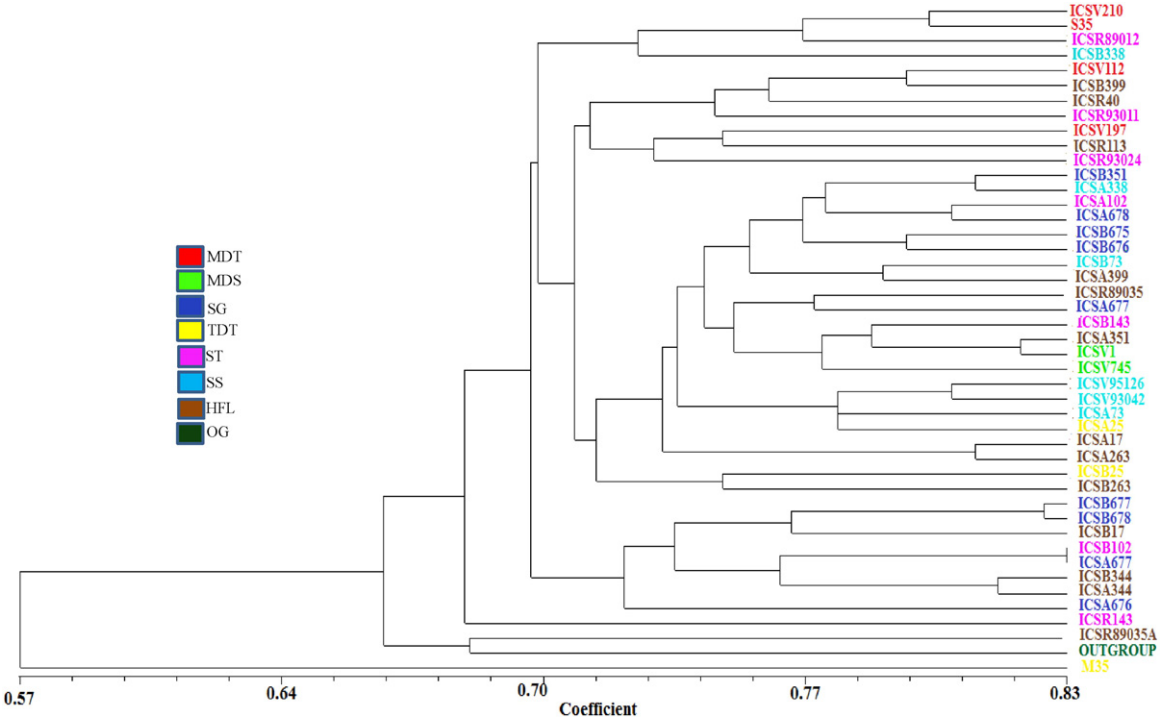

Fig. 1. UPGMA dendrogram of 45 genotypes of sorghum based on Jaccard's similarity coefficient using the cumulative data of ISSR, RAPD and DAMD markers.

the correlation between two datasets of the molecular markers used in the present study using the NTSYS-pc software v. 2.02e (Rohlf, 1998).

3. Results

Fifteen ISSR primers generated totally 132 amplicons out of which 116 were polymorphic with an average of 8.8 amplicons per primer and the amplicons were in the range of 100–3500 bp. The mean percentage of polymorphism revealed by 15 ISSR primers was 86.93%. Eight RAPD primers produced 49 bands, out of which 46 were polymorphic resulting in a very high average polymorphism percentage of 94.09% (Table 2). In DAMD analysis, seven DAMD primers resulted in a maximum number (82) of resolved products than RAPD primers (Table 2). But the mean percentage of polymorphism shown by DAMD primers was relatively lower (72.8%) than those shown by ISSR and RAPD primers. Overall, thirty SPAR markers produced 222 polymorphic amplicons out of 263 amplicons accounting for an average polymorphism percentage of 84.6% with an average PIC value of 0.74 and an average Rp value of 7.16 (Table 2). The representative gel profile for all three markers is given in Fig. S1.

The Mantel's test of correlation revealed that a high measure of correlation (0.70) existed between ISSR and DAMD data, followed by ISSR vs RAPD (0.67) and RAPD vs DAMD (0.58) data. The cumulative data correlated best with the ISSR data having the maximum correlation coefficient value (r) of 0.84 (Table S1). The cumulative data of all three markers were used to construct a UPGMA dendrogram using Jaccard's similarity coefficient (Fig. 1). In dendrogram, mostly the individuals of seven subpopulations failed to form distinct clusters rather intermixed with members of different subpopulations. This indicates that there is less genetic differentiation between subpopulations. However, some of the individuals belonging to the same subpopulations were clustered together signifying their genetic closeness. For instance the members of the MDT (ICSV 210 and S35), SG (ICSB 675 and ICSB 676; ICSB 677 and ICSB 678), SS (ICSV 95126 and ICSV 93042), and HFL (ICSA 17 and ICSA 263; ICSB344 and ICSA 344) subpopulations formed distinguished clusters, thus showing that there is an apparent correlation existing among genotypes with similar agronomic traits/characteristics.

The genetic diversity parameters such as the observed Na and Ne were in the range of 1.18–1.72 and 1.12–1.41 respectively. From this study it was obvious that, the subpopulation HFL had the highest intra population genetic diversity followed by those of SG whereas MDT had the lowest intra population genetic diversity. The values of h, I and Pp where the maximum for HFL subpopulation (0.244, 0.368 and 72.65%, respectively) and the minimum for MDT subpopulation (0.074, 0.109 and 17.95%) (Table 3). For overall populations, the values of h, I and Pp were found to be 0.266, 0.407 and 84.81%, respectively indicating the existence of high genetic variation within populations. The genetic diversity among populations (G_{ST}) and the gene flow (Nm) between populations were observed to be 0.396 and 0.7680 respectively.

The Nei's unbiased measures of genetic identity and genetic distance among seven subpopulations of sorghum have also been calculated (Table S2). Nei's genetic distance between subpopulations was in the range of 0.044 to 0.401. The maximum inter population genetic distance (0.401) was recorded between the genotypes of MDT and OG. The highest value (0.957) of Nei's unbiased measures of genetic identity was observed between the HFL and SG subpopulations while the corresponding genetic distance (0.044) between them was the least (Table S2). AMOVA revealed that there was a significant genetic variation ($P < 0.001$) existing among the seven subpopulations studied (Table 4). Further partitioning of genetic variation showed that a higher extent of genetic variation exists at the intra population level (95%) rather than at inter population (5%) level (Table 4). The results of AMOVA are consistent with the UPGMA cluster analysis.

4. Discussion

A critical and challenging step towards utilization of conserved germplasm is the characterization of the genetic diversity existing within the collection (Casa et al., 2008; Uphadyaya et al., 2009); also progress in plant breeding depends on the extent of genetic variability present in a population. DNA based markers have been widely utilized to orient plant genetic resource conservation management, providing a noteworthy tool to evaluate the genetic diversity, its distribution within and among populations of several crop species and predict the evolutionary potential of those species (Hamrick and Godt, 1997). In the present work, we have compared the efficiency of three different PCR based SPAR methods for analyzing the genetic variation among 45 sorghum genotypes with various agronomic traits. In terms of revealing genetic polymorphism, RAPD with 94% polymorphism surpassed the other two marker systems viz., ISSR (86.9%) and DAMD (72.8%) in this study. Several workers have utilized RAPD markers for the efficient analysis of genetic diversity in sorghum genotypes confirming that RAPD is the most suitable marker for appraising the sorghum genome (Ayana et al., 2000; Menkir et al., 1997). However, in the present study the three methods revealed polymorphism independent of each other from several regions of the genome. Therefore, a combined data analysis of all three methods which consists of more numbers of primers and more coverage of genome together revealed a comprehensive pattern of genetic diversity among the genotypes.

In UPGMA cluster analysis, only few genotypes of same agronomic traits occupied the same clusters while the grouping of rest was not according to their traits. This may be due the fact that the relationships between the genotypes are not necessarily reflecting the agronomic traits. Molecular markers are scattered throughout the genome and their association with various agronomic traits is influenced by the cultivator under selection pressure induced by domestication.

The assessment of genetic diversity within and between populations enables us to infer the shape, inter and intra population genetic structure. In the present study, the low value of coefficient of gene

Table 3
Statistical analysis of genetic diversity and differentiation parameters for seven subpopulations of sorghum genotypes.

Population	Ss	Mean Na ± SD	Mean Ne ± SD	Mean h ± SD	Mean I ± SD	Pp (%)	H_{SP}	H_{POP}	G_{ST}	Nm
MDT	2	1.180 ± 0.385	1.127 ± 0.272	0.074 ± 0.159	0.109 ± 0.232	17.95				
MDS	4	1.474 ± 0.500	1.317 ± 0.390	0.180 ± 0.206	0.295 ± 0.294	47.44				
SG	9	1.637 ± 0.482	1.374 ± 0.378	0.218 ± 0.200	0.328 ± 0.282	63.68				
TDT	3	1.517 ± 0.501	1.349 ± 0.387	0.201 ± 0.206	0.296 ± 0.296	51.73				
ST	9	1.697 ± 0.461	1.411 ± 0.381	0.239 ± 0.197	0.358 ± 0.276	69.66				
SS	5	1.504 ± 0.501	1.327 ± 0.381	0.189 ± 0.204	0.280 ± 0.293	50.43				
HFL	12	1.727 ± 0.447	1.415 ± 0.368	0.244 ± 0.192	0.368 ± 0.269	72.65				
Overall population	45	1.872 ± 0.335	1.442 ± 0.344	0.266 ± 0.173	0.407 ± 0.234	84.81	0.2788	0.1682	0.3967	0.7605

MDT, mid-season drought tolerant; *MDS*, mid-season drought susceptible; *SG*, stay green lines; *TDT*, terminal drought tolerant; *ST*, Saline tolerant; *SS*, Saline susceptible; *HFL*, High Fe–Zn lines.

Ss, Sample size; *Na*, Observed number of alleles; *Ne*, Effective number of alleles; *h*, gene diversity; I, Shannon's information index; Pp, percentage of polymorphic loci; SD, standard deviation; *GST*, diversity among populations; *Nm*, gene flow $0.5(1 - GST) / GST$; H_{pop}, variability within population; *Hsp*, total variability. The diversity parameters were calculated by assuming that the populations are in Hardy–Weinberg equilibrium.

Table 4
Analysis of molecular variance (AMOVA) within and among seven subpopulations of sorghum genotypes.

Source of variation	degrees of freedom (df)	Sum of squares	Mean of squares	Variance components	Percentage of variation
Among populations	6	246.244	41.041	1.546	5%
Within populations	38	1201.489	31.618	31.618	95%
Total	44	1447.733		33.164	100%

differentiation (G_{ST}) (0.396) indicated that there is less genetic differentiation among seven subpopulations of sorghum. This result was further substantiated by AMOVA which showed a higher degree of genetic variation within populations (95%) than among populations (5%) indicating that the accessions are not under selection processes and/or there is a continuous exchange of genes among accessions. Tadesse and Feyissa (2013) also observed a similar trend of higher degree of genetic variation within populations than among populations of sorghum genotypes from Ethiopia using ISSR markers. Several factors influence the genetic structure and diversity of plant populations; these are long-term evolutionary history of the species, genetic drift, mating system, gene flow, and geographic range of the species and species characteristics like long-lived, wind-pollinated and out crossing (Hogbin and Peakall, 1999). The observed pattern of high genetic diversity and low genetic variation among seven subpopulations of sorghum could largely be attributed to wind pollination and out-crossing. The out-crossing plants tend to be more genetically diverse and have less genetic differentiation among populations (Hamrick and Godt, 1996). The highest intra population genetic diversity observed among the members of HFL subpopulation, followed by ST and SG subpopulations could be accounted for the adaptive potential of the plant to withstand harsh environments such as drought and salinity. Thus, in the present study, the documentation of genetic variation in genotypes with various agronomic traits will lead to better utilization of sorghum germplasm by breeders to develop superior varieties with improved agronomic traits.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.gene.2015.10.056.

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