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Genetic Diversity Analysis of Forty Pearl Millet (*Pennisetum glaucum* (L.) R. Br) Accessions from Sudan Using Agronomical Descriptors and DNA Molecular Markers

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Abstract

Morphological descriptors and Random Amplification of Polymorphic DNA (RAPD) technique were used to assess the genetic variation among and within forty *Pennesitum glaucum* accessions from Sudan. Accessions were collected from 30 villages representing Darfur, North Kordofan, South Kordofan, and Blue Nile states. 64 amplified fragments were distinguished using ten primers. 63 bands were polymorphic among the forty accessions with an average of 6.3 polymorphic bands per primer. Low level of genetic similarity was observed (4% - 43%). The PhiPT (analogue of F_{ST} fixation index) value for genetic variability obtained over the four regions was 0.169 with high significance (P = 0.01). AMOVA analysis showed higher variance components within regions (80%) than among regions (20%). The two dendrograms obtained by Random Amplification of Polymorphic DNA (RAPD) data; and morphological data based on 26 descriptors did not fit together. PCA (Principal coordinate's analysis) showed geographic structuring of pearl millet according to its growing regions in Sudan.

Keywords

Millet, Accessions, RAPD, Morphological, Sudan

1. Introduction

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is a cross-pollinated annual C4 crop species that originated in western Africa and was introduced to eastern Africa and Indian sub-continent some years ago [1]. India, China, Myanmar,

Pakistan and Yemen in Asia, and Nigeria, Niger, Burkina Faso, Mali, Sudan, Chad, and Tanzania in Africa are the top countries in each region producing pearl millet grain [2].

In the western Sudan (Kordofan and Darfur), pearl millet is the stable food for the majority. For cultivation area and total production, it comes second to sorghum [3]. Sudan is the center of origin for pearl millet as it is a part of the Sahel zone which extends from Western Sudan to Senegal. About 18 species of Pennisetum are found in the country [4]. The National Gene Bank collection currently contains 1017 (pearl) millet accessions. They are maintained by the Agricultural Research Corporation in Sudan. Recently, new technologies at low scale adopting the DNA based methods were initiated [4]. Morphological characterization was the first method used by researchers to select superior genotypes, spring wheat [5], barn swallows [6], and maize [7] [8] and [9].

However, several studies showed that morphological markers are not suitable for traits with low heritability and are highly affected by environments [6] [10] [11]. These limitations have led to the evolution of molecular markers. Molecular markers can serve not only as an important tool to help in distinguishing between germplasm accessions, but also to characterize and determine genetic distance among groups of genotypes [12]. Recently, many molecular markers were used to determine the genetic relation between millet germplasms. Few researches have been done to investigate the genetic variations present in the Sudanese millet genotypes. Therefore, the main objectives of this study were to assess the genetic variation among and within forty millet accessions collected from different regions in Sudan using morphological characterization and the Random Amplified Polymorphic DNA (RAPD) technique, to study the pattern of regional genetic diversity of pearl millet, by analyzing accessions for the four studied states, and to find out whether there is a relationship between the phenotypic and genotypic characterization of studied millet accessions.

2. Materials and Methods

2.1. Plant Material

Forty millet accessions evaluated in this study were provided by the Germplasm of the Genetic Resource Unit of the Agricultural Research Corporation, Wad Medani. The seeds were collected from 30 villages from different regions of Sudan, namely: 1) North Kordofan, 2) South Kordofan, 3) Blue Nile State, and 4) Darfur (**Table 1**). Two Sorghum genotypes (Tabat and Wad Ahmed) were also included in the study for comparison.

2.2. DNA Extraction

The DNA extraction was done by CTAB 2X method. Genomic DNA was extracted from fresh leaf tissues using modified CTAB method [13]. In this method the fine powdered plant materials were immediately transferred into 13 ml Falcon tubes containing 6 ml of pre-warmed lysis solution. Tubes containing the

Table 1. The pearl millet accessions used in the study and their abbreviations based on collection site.

	Accession No.	Accession abbreviation		Accession No.	Accession abbreviation
1	HSD 3509	NI	21	HSD 6080	S21
2	HSD 3510	N2	22	HSD 6081	S22
3	HSD 3512	N3	23	HSD 6082	S23
4	HSD 3517	N4	24	HSD 2369	S24
5	HSD 3522	N5	25	HSD 5536	B25
6	HSD 3523	N6	26	HSD 5546	B26
7	HSD 3524	N7	27	HSD 5551	B27
8	HSD 3525	N8	28	HSD 5558	B28
9	HSD 3528	N9	29	HSD 5560	B29
10	HSD 4263	S10	30	HSD 5564	B30
11	HSD 4265	S11	31	HSD 5566	B31
12	HSD 4269	S12	32	HSD 5574	B32
13	HSD 4271	S13	33	HSD 5581	B33
14	HSD 4272	S14	34	HSD 2090	D34
15	HSD 4277	S15	35	HSD 2132	D35
16	HSD 4283	S16	36	HSD 2150	D36
17	HSD 4285	S17	37	HSD 2157	D37
18	HSD 4286	S18	38	HSD 2168	D38
19	HSD 6073	S19	39	HSD 2223	D39
20	HSD 6076	S20	40	HSD 2245	D40

N = North Kordofan, S = South Kordofan, B = Blue Nile State, D = Darfur.

samples were then incubated in water path at 65˚C with gentle shaking for 30 minutes and left to cool at room temperature for 5 min. Isoamyl alcohol and chloroform mixture (1:24) was added to each tube and the phases were mixed gently for 5 min. at room temperature to make a homogenous mixture. The cell debris was removed by centrifugation at 5000 rpm for 15 min at room temperature and the resulted clear aqueous phases (containing DNA) were transferred to new sterile tubes. The step of the chloroform: Isoamyl alcohol extraction was repeated twice. The nucleic acid in the aqueous phase was precipitated by adding equal volume of deep cooled isopropanol. The contents were mixed gently and collected by centrifugation at 4000 rpm for 10 min. Then the formed DNA pellet was washed twice with 70% ethanol and the ethanol was discarded after spinning with flash centrifugation the remained ethanol was removed by leaving the pellet to dry at room temperature. The pellet was dissolved in TE buffer and stored at −20˚C for further use. The extracted DNA samples were observed under UV illumination after staining with ethidium bromide and agarose gel electrophoresis.

2.3. RAPD Analysis and Primer Selection

Twenty primers were used for PCR amplification, ten primers that produced strongly amplified polymorphic bands with these test templates were selected for RAPD-PCR analysis (Table 2). The PCR reaction mixtures were prepared in 25 µl volumes containing 2.5 µl of 10X Taq buffer, 1.5 µl $MgCl_2$ (50 mM), 2.5 µl dNTPs (2 Mm/µl), 2 µl random primer (10 pmol/ µl), 0.5 µl Taq DNA polymerase (5 U/µl) and 1 µl of the extracted DNA (10 ng). The mixture was made up to 25 µl by addition of DDH_2O (double sterilized distilled water). RAPD/PCR reactions were optimized and initiated using an applied Biometra thermalcycler programmed to repeat the thermal profile. Setting of the PCR program is based on three steps. Step one, was an initial denaturation step at 94˚C for 5 mins. Step two, was run for 40 cycles, each starting with denaturation at 94˚C for 1 min, followed by annealing 36˚C for 1 min and ended by extension at 72˚C for 1 min. Step three, was a final extension cycle performed at 72˚C for 7 minutes .The PCR machine was adjusted to hold the product at 4˚C. The PCR product was mixed with 3 µl of loading dye and spun briefly in a micro centrifuge before loading. The PCR products and 1 kb DNA ladder were electrophoresed using 2% agarose gel at 100 volts followed by ethidium bromide then separated fragments and were visualized with ultraviolet (UV) transilluminator (Figure 1).

2.4. Analysis of Genetic Diversity

Each band in the RAPDs profile was treated as an independent locus with two alleles. The numbers of bands produced for each primer were scored manually for presence (1), or absence (0) and a binary matrix was generated and then used for further analysis [14]. The PhiPT (analogue of F_{ST} fixation index) value for genetic variability was calculated for the four regions using GenALEx v.6.1 [15]. With AMOVA, the variance components and their significance levels for variation

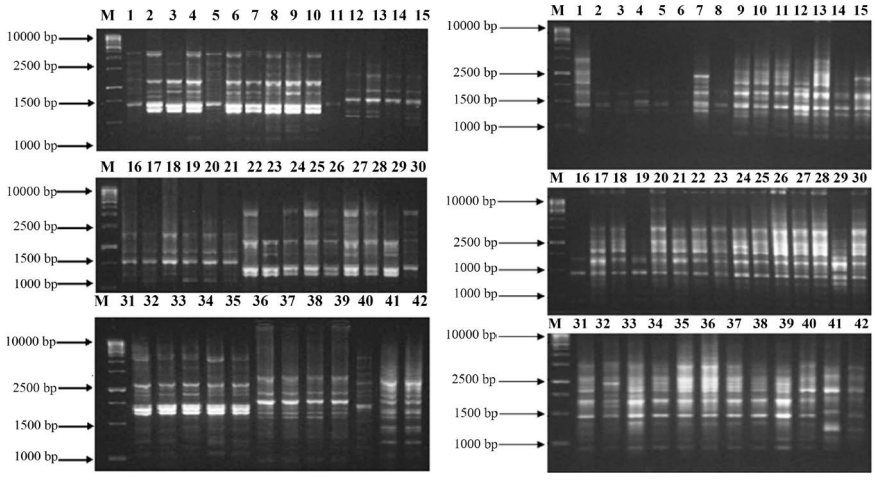

Figure 1. RAPD amplification patterns with primers D-8 and UBC-104 (from left to right: M = ladder, lanes 1 to 40 represent the millet accessions studied, and lanes 41 and 42 are sorghum genotypes.

Table 2. Polymorphism detected by the use of ten polymorphic random primers on forty *Pennisetum glaucum* accessions.

Name of primer	Sequence of primer (5' to 3')	Total number of bands	Number of polymorphic bands	Number of monomorphic bands	% of polymorphic bands
A-1	CAG GCC CTT C	7	7	0	100
B-8	GTC CAC ACG G	10	10	0	100
B-20	GGA CCC TTA C	9	9	0	100
C-2	GTG AGG CGT C	6	6	0	100
C-10	TGT CTG GGT G	6	6	0	100
D-8	GTG TGC CCC A	6	6	0	100
UBC-101	GCG GCT GGA G	4	3	1	75
UBC-104	GGG CAA TGA T	5	5	0	100
UBC-122	GTA GAC GAG C	5	5	0	100
UBC-155	CTG GCG GCT G	6	6	0	100
Total		64	63	1	975
Average		6.4	6.3	0.1	97.5

among regions and within regions were obtained by using RAPD data of the 40 accessions (**Table 3**) and (**Figure 2**).

2.5. Genetic Structure

A principal coordinate analysis (PCA) was conducted with GenALEx v.6.1. This multivariate approach was chosen to complement the cluster analysis information (**Figure 3**), because cluster analysis is more sensitive to closely related individuals whereas PCA is more informative regarding distances among major groups [16].

Pairwise genetic distance between individuals were calculated by the percentage disagreement method (**Table 4**). These data were used in cluster analysis with the unweighted pair-group method using arithmetic averages (UPGMA), in which samples were grouped based on their similarity (**Figure 4**) with the aid of statistical software package STATISTCA-ver.6 [17].

2.6. Agronomical Field Study

Phenotypic diversity and agronomic descriptors for the 40 millet accessions were also recorded based on 26 agronomical descriptors for each accession. These observed data were taken during the season 2006-2007 (**Table 5**).

2.7. Statistical Analysis of Agronomical Descriptors

Analysis of variance was done using the SPSS version 17 computer software [18] program in order to assort accessions according to their agronomic characters (**Figure 5**).

Table 3. Summary of the analysis of molecular variance (AMOVA) within and among the four *Pennisetum glaucum* regions in Sudan.

Source	Df	SS	MS	Est. Var.	%	Stat	Value	Probability
Among regions	3	87.584	29.195	2.116	20%			
Within regions	36	312.216	8.673	8.673	80%	PhiPT	0.196	0.010
Total	39	399.800		10.788	100%			

The analysis is based on RAPD phenotypes consisting of 63 bands states.

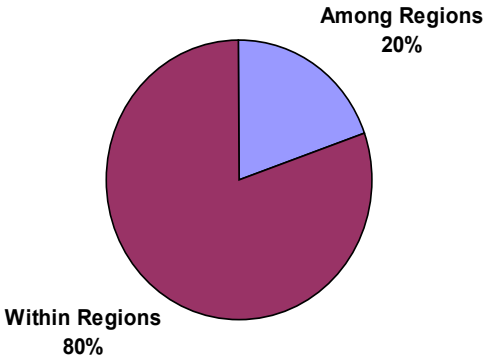

Figure 2. Percentages of molecular variance within and among the four *Pennisetum glaucum* regions based on 63 RAPD loci.

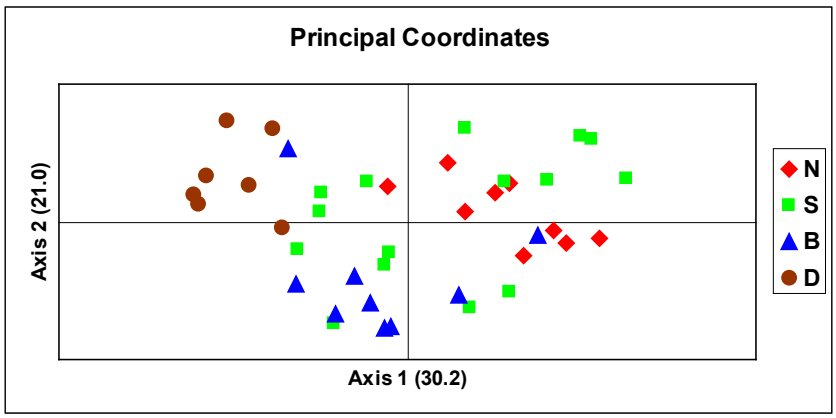

Figure 3. Principal coordinates analysis for the four *Pennisetum glaucum* regions. Note the separation between Darfur individuals and the other three regions; where N = North Kordofan, S = South Kordofan, B = Blue Nile and D= Darfour.

3. Results

3.1. Amplification of RAPD Primers

Ten primers which were informative (UBC-101, UBC-104, UBC-122, UBC-155, A-1, B-8, B-20, C-2, C-10, D-8) were selected and used to evaluate the degree of polymorphism and genetic relationship within and between all individuals under study (Table 2). The selected primers generated an appropriate amplification pattern with clear and consistent reproducible bands. The maximum

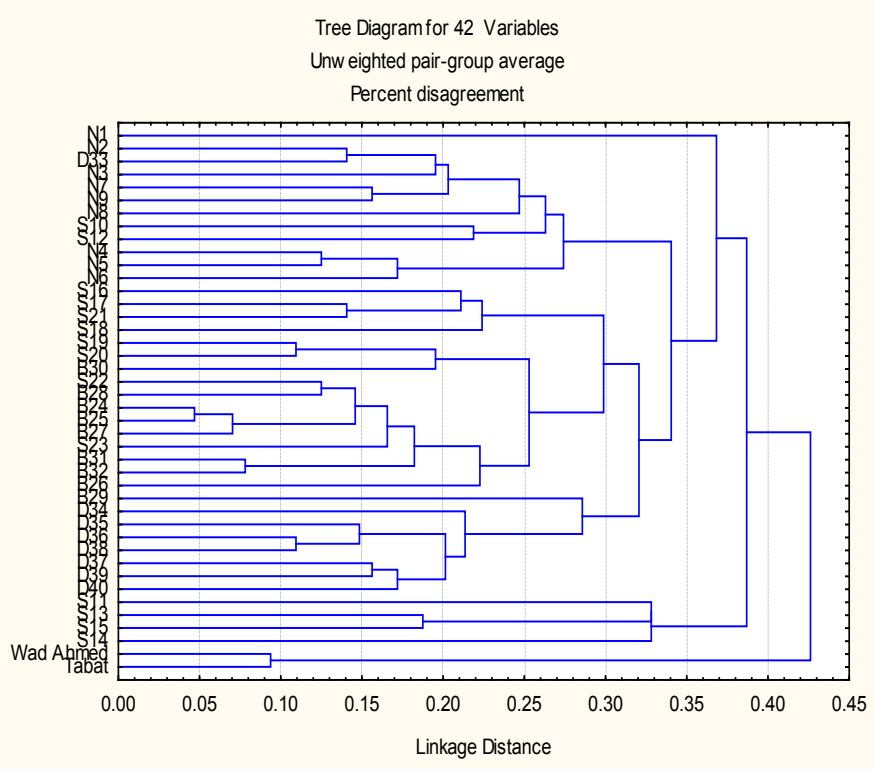

Figure 4. Dendrogram based on UPGMA clustering using the percentage disagreement, showing relationships of 40 pearl millet accessions of and two genotypes of Sorghum. Where N = North Kordofan, S = South Kordofan, B = Blue Nile and D = Darfour.

Table 4. Matrix of RAPD dissimilarity among 40 *Pennisetum glaucum* Accessions based on Nei and Lei coefficients.

	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
1	0.00																																									
2	0.42	0.00																																								
3	0.33	0.19	0.00																																						
4	0.38	0.17	0.27	0.00																																					
5	0.34	0.27	0.33	0.13	0.00																																				
6	0.41	0.17	0.20	0.19	0.16	0.00																																		
7	0.31	0.20	0.20	0.31	0.38	0.25	0.00																																	
8	0.36	0.25	0.25	0.23	0.33	0.33	0.23	0.00																																
9	0.31	0.20	0.20	0.34	0.38	0.25	0.16	0.20	0.00																															
10	0.30	0.28	0.25	0.23	0.27	0.23	0.23	0.31	0.23	0.00																														

Continued

11	0.38	0.33	0.33	0.34	0.31	0.41	0.34	0.33	0.25	0.33	0.00																								
12	0.30	0.28	0.25	0.30	0.30	0.27	0.23	0.34	0.23	0.22	0.33	0.00																							
13	0.36	0.28	0.38	0.30	0.30	0.33	0.33	0.44	0.30	0.31	0.30	0.22	0.00																						
14	0.39	0.47	0.38	0.33	0.33	0.42	0.36	0.31	0.39	0.34	0.33	0.28	0.38	0.00																					
15	0.39	0.38	0.38	0.36	0.39	0.36	0.36	0.44	0.27	0.31	0.36	0.22	0.19	0.28	0.00																				
16	0.34	0.36	0.33	0.47	0.47	0.41	0.25	0.36	0.22	0.39	0.28	0.39	0.39	0.45	0.42	0.00																			
17	0.34	0.39	0.36	0.38	0.41	0.41	0.28	0.33	0.31	0.39	0.34	0.39	0.45	0.33	0.42	0.19	0.00																		
18	0.44	0.36	0.36	0.34	0.38	0.41	0.38	0.23	0.38	0.42	0.34	0.45	0.48	0.36	0.52	0.22	0.22	0.00																	
19	0.36	0.31	0.34	0.30	0.30	0.30	0.30	0.41	0.33	0.41	0.36	0.38	0.34	0.41	0.38	0.30	0.23	0.33	0.00																
20	0.38	0.23	0.30	0.25	0.25	0.25	0.31	0.36	0.28	0.33	0.31	0.33	0.33	0.42	0.39	0.31	0.28	0.31	0.11	0.00															
21	0.33	0.34	0.34	0.36	0.36	0.39	0.27	0.31	0.27	0.38	0.30	0.38	0.38	0.31	0.38	0.23	0.14	0.23	0.13	0.20	0.00														
22	0.34	0.39	0.36	0.28	0.34	0.34	0.28	0.36	0.34	0.27	0.34	0.30	0.39	0.33	0.36	0.38	0.28	0.41	0.33	0.34	0.30	0.00													
23	0.39	0.34	0.31	0.30	0.36	0.39	0.27	0.28	0.33	0.34	0.36	0.38	0.41	0.34	0.41	0.36	0.20	0.30	0.28	0.30	0.19	0.20	0.00												
24	0.38	0.30	0.33	0.25	0.31	0.34	0.25	0.27	0.31	0.36	0.34	0.39	0.39	0.39	0.45	0.34	0.25	0.34	0.23	0.25	0.20	0.16	0.14	0.00											
25	0.36	0.31	0.31	0.27	0.33	0.33	0.23	0.28	0.30	0.38	0.36	0.41	0.41	0.41	0.44	0.33	0.27	0.39	0.25	0.27	0.25	0.17	0.19	0.05	0.00										
26	0.34	0.36	0.33	0.34	0.41	0.41	0.25	0.33	0.31	0.42	0.34	0.36	0.42	0.45	0.48	0.38	0.34	0.38	0.33	0.34	0.30	0.25	0.23	0.19	0.17	0.00									
27	0.36	0.28	0.31	0.20	0.27	0.27	0.23	0.31	0.33	0.31	0.36	0.38	0.38	0.38	0.41	0.39	0.27	0.36	0.19	0.20	0.22	0.17	0.16	0.08	0.06	0.17	0.00								
28	0.38	0.33	0.33	0.28	0.34	0.34	0.25	0.27	0.28	0.30	0.34	0.39	0.42	0.39	0.42	0.28	0.25	0.28	0.27	0.28	0.23	0.13	0.14	0.09	0.14	0.25	0.14	0.00							
29	0.52	0.44	0.38	0.45	0.45	0.42	0.36	0.34	0.39	0.41	0.42	0.38	0.50	0.41	0.44	0.45	0.36	0.36	0.47	0.45	0.41	0.36	0.22	0.36	0.38	0.39	0.34	0.30	0.00						
30	0.38	0.33	0.36	0.28	0.28	0.34	0.34	0.36	0.28	0.33	0.31	0.36	0.30	0.42	0.33	0.31	0.34	0.38	0.17	0.22	0.23	0.25	0.30	0.22	0.23	0.28	0.20	0.19	0.45	0.00					
31	0.44	0.23	0.27	0.22	0.31	0.25	0.25	0.30	0.28	0.30	0.41	0.36	0.42	0.45	0.45	0.34	0.31	0.31	0.23	0.22	0.30	0.22	0.23	0.16	0.20	0.28	0.17	0.13	0.33	0.22	0.00				
32	0.42	0.25	0.25	0.23	0.36	0.30	0.23	0.28	0.27	0.28	0.39	0.41	0.44	0.50	0.47	0.33	0.33	0.33	0.22	0.20	0.28	0.23	0.25	0.14	0.16	0.23	0.16	0.14	0.41	0.20	0.08	0.00			
33	0.41	0.14	0.20	0.25	0.31	0.22	0.22	0.30	0.19	0.23	0.34	0.27	0.33	0.45	0.33	0.38	0.38	0.38	0.30	0.25	0.27	0.34	0.30	0.31	0.36	0.34	0.30	0.28	0.39	0.28	0.22	0.23	0.00		
34	0.42	0.31	0.28	0.30	0.39	0.36	0.33	0.28	0.30	0.38	0.45	0.44	0.47	0.47	0.44	0.33	0.33	0.33	0.38	0.33	0.34	0.33	0.22	0.27	0.25	0.33	0.28	0.20	0.34	0.30	0.17	0.22	0.30	0.00	
35	0.30	0.41	0.38	0.33	0.42	0.48	0.36	0.28	0.36	0.34	0.45	0.41	0.44	0.44	0.47	0.36	0.36	0.39	0.44	0.45	0.38	0.30	0.28	0.27	0.28	0.33	0.34	0.23	0.38	0.33	0.27	0.31	0.39	0.19	0.00

Continued

36	0.34	0.42	0.36	0.41	0.44	0.50	0.34	0.30	0.38	0.45	0.41	0.42	0.48	0.45	0.48	0.31	0.28	0.31	0.36	0.41	0.30	0.31	0.20	0.22	0.23	0.28	0.30	0.19	0.30	0.34	0.28	0.30	0.41	0.23	0.14	0.00					
37	0.39	0.47	0.34	0.39	0.42	0.48	0.36	0.31	0.39	0.41	0.42	0.41	0.50	0.34	0.44	0.36	0.33	0.33	0.47	0.45	0.38	0.39	0.25	0.36	0.34	0.36	0.34	0.30	0.22	0.45	0.36	0.41	0.45	0.22	0.25	0.23	0.00				
38	0.39	0.41	0.34	0.33	0.36	0.45	0.36	0.28	0.39	0.41	0.42	0.44	0.50	0.38	0.50	0.33	0.27	0.27	0.38	0.39	0.28	0.30	0.19	0.23	0.25	0.33	0.28	0.17	0.28	0.36	0.23	0.28	0.36	0.19	0.16	0.11	0.16	0.00			
39	0.39	0.38	0.31	0.42	0.42	0.39	0.30	0.34	0.30	0.34	0.42	0.42	0.31	0.47	0.44	0.27	0.30	0.33	0.44	0.39	0.38	0.36	0.31	0.34	0.34	0.39	0.38	0.27	0.22	0.42	0.30	0.34	0.36	0.25	0.20	0.23	0.16	0.16	0.00		
40	0.31	0.42	0.36	0.38	0.41	0.44	0.23	0.31	0.39	0.41	0.42	0.41	0.55	0.36	0.52	0.25	0.19	0.22	0.36	0.38	0.30	0.31	0.27	0.28	0.27	0.31	0.30	0.22	0.27	0.34	0.25	0.30	0.41	0.20	0.20	0.19	0.17	0.14	0.17	0.00	
41	0.39	0.47	0.38	0.42	0.52	0.52	0.42	0.38	0.48	0.41	0.39	0.38	0.50	0.38	0.47	0.48	0.45	0.45	0.56	0.55	0.50	0.45	0.44	0.48	0.50	0.48	0.47	0.45	0.38	0.58	0.45	0.50	0.48	0.41	0.38	0.39	0.25	0.38	0.38	0.36	0.00
42	0.39	0.38	0.34	0.36	0.45	0.48	0.39	0.31	0.45	0.38	0.42	0.34	0.50	0.38	0.47	0.45	0.48	0.39	0.50	0.48	0.42	0.41	0.41	0.45	0.50	0.48	0.47	0.39	0.38	0.52	0.39	0.44	0.39	0.38	0.34	0.36	0.25	0.31	0.34	0.36	0.09

Table 5. The 26 morpho-agronomical parameters taken from field experiment during season 2006-2007.

Sample No	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
N1	228	4.4	3	6	1	3	4	3	3	4	4	2	68	4	3	2.5	25.5	3	2	3	4	9	2	3	4	9.1
N2	222	4.5	3	5	3	3	4	4	4	3	4	2	81	4	3	1.5	28.5	2.5	2	2	4	10	3	3	4	9
N3	219	5	2	6	2	4	4	3	3	4	4	3	76	4	3	4.6	31	3.5	5	2	4	11	2	3	4	8.4
N4	211	3.2	2	6	2	2	2	4	4	3	2	2	60	4	3	16.3	26.5	2.9	7	2	4	8	3	3	4	9.1
N5	238	4.4	3	3	1	4	4	3	4	3	4	2	69	4	3	7.5	35.3	3.5	5	2	3	12	2	3	4	10.8
N6	207	3.5	2	4	1	3	3	4	4	3	4	2	77	2	3	1	31.5	3	5	3	4	9	2	4	4	10.4
N7	218	5	3	3	1	2	3	3	4	4	4	2	71	4	3	10.5	29	3	7	3	2	11	2	3	4	12.3
N8	233	4	2	5	2	4	4	3	3	3	2	2	62	2	3	4	33.5	3.8	7	3	4	12	2	3	4	12.3
N9	221	5.4	3	4	2	3	3	3	4	3	4	2	69	4	3	3.5	25	3.5	7	4	2	12	2	3	4	11.4
S10	235	5	3	5	1	3	4	4	4	4	4	2	74	2	3	10.3	23	2.9	2	2	2	12	2	4	4	8
S11	210	4	4	5	1	3	4	4	4	3	4	2	65	4	3	5	31	2.8	1	3	4	12	2	3	4	12.3
S12	253	4.4	2	3	1	4	4	4	4	3	4	2	77	2	3	4.5	36.5	2.3	3	2	2	9	2	3	4	8.6
S13	200	3.4	2	2	2	4	4	3	4	4	4	2	77	4	3	8.4	24	3	2	3	4	11	2	3	4	13.6
S14	282	4.4	3	3	2	4	4	3	4	3	4	2	76	4	3	11.5	26	2.5	1	3	2	13	2	3	4	10.5
S15	267	5	3	3	1	2	4	3	3	4	3	3	77	4	3	16.5	32	3.3	3	3	2	12	2	3	4	12
S16	185	4.4	2	4	1	3	3	4	3	3	4	3	70	4	3	8	25	2	3	2	4	9	3	3	4	8.5
S17	200	3	3	3	1	2	4	4	4	3	3	4	82	4	4	0	32	4	3	2	2	12	2	3	4	7.6
S18	231	5	3	5	2	3	3	4	3	3	3	2	80	4	3	4.5	33	2.3	3	4	3	11	2	4	4	9.8
S19	212	5.4	2	4	2	2	3	3	3	3	3	2	80	4	3	3.5	26	3.3	2	3	2	10	2	3	4	8.5
S20	237	4	2	3	1	3	3	4	3	4	4	2	68	4	3	5.4	31	2	3	2	4	11	2	3	4	9.1
S21	279	4	3	6	2	2	3	3	4	3	4	2	85	3	3	4	33	2.8	3	2	4	13	3	3	4	7.3
S22	279	6	4	4	1	2	4	4	3	3	3	2	72	4	4	0	35.5	3.5	3	3	2	14	2	3	3	9.3
S23	205	5	3	4	2	2	4	3	4	4	4	2	80	4	3	6	32	3	3	3	2	11	2	3	3	8.2
S24	266	5.4	3	3	1	2	4	4	4	3	3	2	82	4	4	0	33	2.5	3	3	4	14	2	3	4	9
B25	289	4.4	2	2	2	3	3	3	4	3	4	2	80	4	3	5.5	28.5	2.5	1	3	2	11	2	3	3	11.6
B26	237	4	2	3	2	2	4	2	4	3	4	3	77	3	4	0	31	2.5	2	2	4	11	3	3	4	8.4
B27	238	4	3	4	1	2	4	4	4	3	4	2	77	4	3	5.5	37	3.6	4	3	4	12	2	3	4	9.1

Continued

B28	237	4	3	2	1	4	3	4	4	4	4	2	67	4	3	13.8	32	2.5	1	3	4	12	2	3	4	11.8
B29	305	4.4	3	2	2	2	3	3	4	3	4	2	76	4	3	1.8	34	2.5	1	4	2	11	3	3	4	10.2
B30	215	4.4	3	4	2	2	3	4	3	4	3	2	67	4	3	6.4	28	2.8	7	3	2	11	2	3	4	10.3
B31	179	5	2	3	2	4	3	4	4	3	4	2	70	3	3	10.9	35	3.5	3	3	4	10	2	4	4	11.8
B32	194	4	2	2	1	4	2	4	3	4	4	4	70	2	3	8,4	24	3	1	2	4	12	3	3	4	15.1
B33	217	4.4	2	2	2	2	3	2	3	3	3	3	65	4	3	5	31	2.5	2	3	4	10	3	3	4	14.3
D34	234	4.4	4	2	2	2	3	4	4	3	4	2	68	3	3	9.5	28.5	2.5	5	3	2	7	3	3	3	9
D35	255	4.4	4	2	2	2	4	4	4	4	4	2	75	4	3	10.4	28.5	3.8	6	2	4	13	3	3	4	13.5
D36	200	4	2	5,6	2	3	4	4	3	4	3	2	75	4	3	0.3	15,22	3,2.5	7	3	3	10	3	3	4	10.6
D37	237	5	4	6	2	3	3	4	3	3	1	2	65	4	3	9	29	2	1	4	2	10	4	3	4	9.5
D38	270	3.4	3	2	1	4	4	3	4	4	4	2	77	4	3	1	22.5	3.3	1	3	4	15	2	3	4	9.1
D39	257	4.4	4	5	2	3	2	4	4	4	3	2	77	4	4	10	35.5	3.3	6	4	2	15	3	3	4	9.1
D40	255	4	3	5	2	2	3	4	4	3	4	2	83	4	4	5	33	3	3	3	4	13	2	3	4	9

A = Plant height (cm), B = Stem diameter (cm), C = Early vigour, D = Total no. of tillers, E = no. of productive tillers, F = No. of Nodal tillers, G=Plant aspect, I = Green fodder yield potential, J = Spike shape, K = Spikelet shattering, L = Bristle length, M = Days to 50% flowering, N = Flowering range, O = Ear exertion type, P = Ear exertion distance (cm), Q = Spike length (cm), R = Spike thickness (cm), S = Seed color, T = Seed covering, U = Seed shape, V = No. of leaves, W = Senescence, X = Juice quality, Y = Stalk Juiciness, Z = 1000 Seed weight (g).

number of fragments was produced by the primer B-8 (10 bands) with 100% polymorphism while the minimum number of fragments was produced by the primer UBC-101 (4 bands) with 75% polymorphism. A total of 64 amplified fragments were distinguished across the selected primers and the statistical analysis showed 63 polymorphic bands among the 40 accessions with an average of 6.3 polymorphic bands per primer and only one monomorphic band with an average 0.1. The number of bands per primer ranged from 4 to 10 and the number of polymorphic bands per primer varied from 3 to 10. All the primers which were used in this study were 100% polymorphic, except primer UBC-101 which was 75% polymorphic (Table 2).

The analysis of molecular variance (AMOVA) for the four regions showed significant difference ($P < 0.01$), with 80% of the differentiation attributed to within regions and 20% attributed to among regions (Figure 2).

The PhiPT estimates were moderate to high (0.196) and significant ($P = 0.01$) (Table 3). The UPGMA and percent disagreement values (PDV) were used to estimate the degree of relationships between the individuals analyzed based on common amplified fragments. Based on the matrix obtained (Table 4), the percent disagreement values (PDV) for all individuals was ranging from 4% to 43%. Principal components analysis of molecular variance performed in GenAlex 6.1 was based on individual banding pattern.

3.2. Agronomical Analysis

The cluster analysis based on Euclidean distance (Figure 5) using agronomical characters grouped the forty pearl millet accessions into two main clusters. B32 was alone in a separate arm as the most divergent accession. Cluster one included

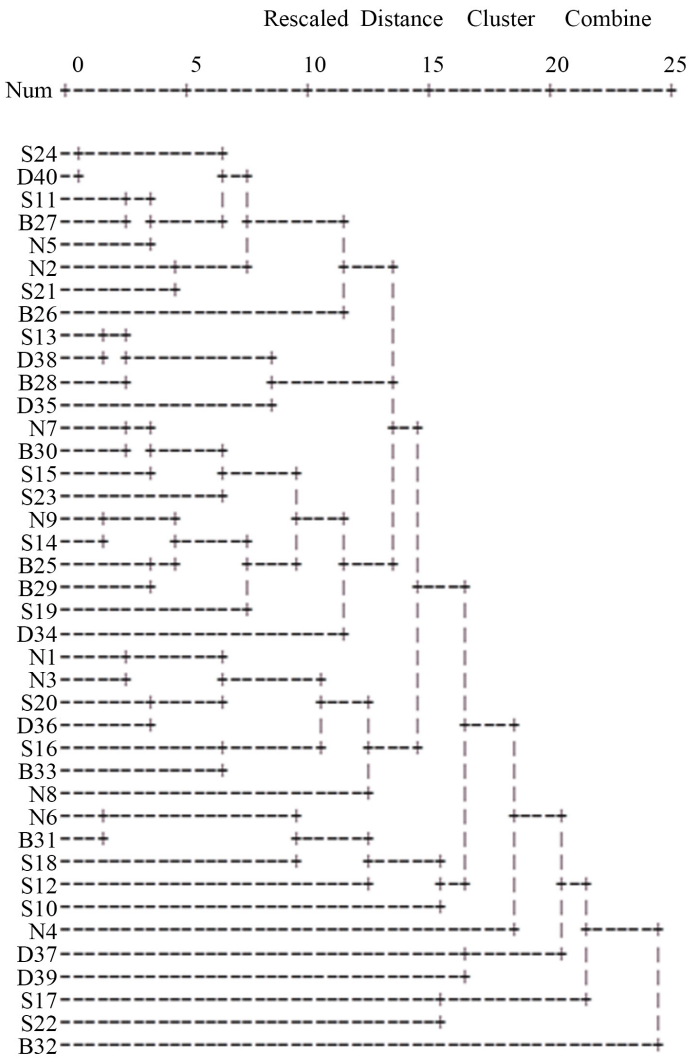

Figure 5. Cluster diagram based on SPSS analysis for the 40 pearl millet accessions classified using 26 agronomic characters.

two accessions S17 and S22, cluster two divided into two subgroups, subgroup one includes two accessions and subgroup two divided into many sub-clusters. The evaluation of pearl millet accessions based on the 26 agronomical descriptors had shown that there is a high amount of variation among the accessions.

4. Discussion

4.1. Molecular Analysis

The genetic similarity obtained in this study ranged from 4% to 43%. These results are in agreement with other results reported by [12] who used 32 RAPD primers for 11 pearl millet accessions from different commercial agricultural areas (the Cerrados region of Brazil). They found that polymorphism among the pearl millet accessions tested, ranged from 9% to 76%. In the first cluster of the dendrogram, it is clear that millet accessions from Darfour except D33, Blue Nile

and most millet accessions from South Kordofan grouped together. However, within this group, Darfour millet genotypes are clearly distinct as they fall in a separate sub-group and accessions from Blue Nile and South Kordofan fall in another sub group. All North Kordofan accessions fall in a second group containing only two accessions from South Kordofan and one accession from Darfour. The close genetic similarity which exhibited by accessions from different regions (Blue Nile and Darfur, Blue Nile and South Kordofan) might be as a result movement of seeds among these regions. The fact that it is very difficult for farmers to keep their own selected seeds and the consequent substantial seed exchanges between them is probably the main factor accounting for the genetic homogeneity which has been observed in this study. South Kordofan accessions were the most heterogeneous genotypes based on their scattered positions in the dendrogram and their highest percentage of polymorphic (88.2%). Whereas, Darfour genotypes were the most distinct millet accessions. So results from this study can help breeding programs and samples selections can become very easy, quick and useful for the millet breeders. The genetic "similarity" between the significant fraction of the South Kordofan pearl millet accessions and the Blue Nile accessions could illustrate a preferential seed flow. It is likely that South Kordofan region is a provider of seed lots to Blue Nile region that can be approved by the higher polymorphism found in South Kordofan region (88.2%), whereas Blue Nile maintains (68.7%) as shown from the regional diversity analysis. This illustrates a significant seed flow. PhiPT and F_{ST} are analogous standardizing measures of the degree of genetic differentiation among regions: scores for both measures range from 0 (non differentiation) to 1 (non alleles shared). The Principle Coordinate Analysis for the four *Pennisetum glaucum* regions showed clear scattering of the South Kordofan genotypes and all the other three regions. However, it was also clear that Darfur genotypes remained intact in a distant group. In this study, the PhiPT value obtained over all regions was (0.196) with high significance ($P = 0.01$), suggesting tendency to high structuring within the region. There was genetic variation of 80% within the regions and the estimated genetic variation was 20% among them. Every region still maintains its indigenous genetic material so that these farmers keep their own seeds sources. So the pattern that is depicted by the PCA analysis is in consistence with the UPGMA tree, the PCA revealed the real regions structure, Darfour grouped separately in the dendrogram and in the PCA graph. The high proportion of variation attributable to within the region (80%), in this study was consistent with the expected population structure for an out crossing species. For pearl millet, it is expected that the heterogeneity and heterozygosity at many loci within a landrace accession to be high. This is due to cross-pollinated nature of the crop. The self-pollinated species such as *Hordeum spontaneum* has 43% of within population variation [19]. In contrast, cross-pollinated species has been reported to have 72% - 100% of their total variation [20] [21]. Our results are in agreement with the findings of [22], who studied genetic diversity of pearl millet

in Niger by using AFLP marker. They found that most of the diversity was found at the population level. Also, a previous study done by [23] in India and by [24] in Nigeria reported 88% to 98% of the variation was found between individuals within populations. They concluded that the high diversity found within a population may be due to out-breeding reproduction system of pearl millet as well as from the seed flow observed at the regional level. Previous studies on pearl millet from Africa and India based on isozymes diversity indicated intra-population diversity of 70% - 90% of the total diversity, depending on their regions of origin [25] [26]. Generally, RAPD technique in this study was very useful. The study provided very useful knowledge about genetic variability of some *Pennisetum glaucum* accessions from Sudan which may allow more efficient and effective uses of resources in plant improvement programs. In this study, RAPD technique was utilized for better assessment of *Pennisetum glaucum* accessions relationship collected from four regions of Sudan. The genetic dissimilarity values obtained with RAPD have been introduced for measuring genetic relationships in many plant species for easiness of the method, which only requires PCR technology. This study clearly showed that it was possible to analyze the RAPD and for correlating their similarity and distance between *Pennisetum glaucum* accessions. From the results of RAPD profiling, it was observed that *Pennisetum glaucum* accessions produced very good amplification with the primers used. Molecular markers can be used to study the genetic diversity and genetic relationships among *Pennisetum glaucum* accessions at the DNA level. The present investigation of DNA profiling in *Pennisetum glaucum* species clearly demonstrates that it is possible to analyze the RAPD patterns for correlating their similarity and distance between accessions. Although accessions from Darfour had the least percentage of polymorphic fragments (60.3%) from all the other accessions, they were grouped together in both the dendrogram and the PCA analysis. This shows that, these accessions might maintain alleles (genes) that are unique to the region. North Kordofan had the second least percentage of polymorphic loci (62.9%). These accessions as well grouped together and were distant from those of Darfour. Although accessions from South Kordofan had the highest percentage of polymorphic loci (88.2%), they were dispersed with groups of North Kordofan and Blue Nile. The analyses of RAPD revealed high levels of genetic variability, even with the use of limited set of primers. This high level of polymorphism among individuals suggests that RAPD techniques can be useful for *Pennisetum glaucum* for the maintenance of Germplasm banks and the efficient selection of parents for breeding.

4.2. Agronomical Analysis

The results showed that B32 was least similar to others, and seems to be a different millet genotype. S22 was the second least similar genotype to other accessions. From the two dendrograms, the cluster based on data obtained from RAPD markers and the cluster based on the agronomical descriptors, showed no

similarity between the two results. The accessions were distributed in different sites in both clusters, so the classification based on RAPD markers could not be related to agronomic descriptors about the accessions in this study. These results are in agreement with those results reported by [27] they studied complex samples in two pearl millet fields in two villages of southwestern Niger, by using both morphological and AFLP marker. Also the results are in agreement with [28] they studded diversity in 21 cotton genotypes by using RAPD marker and morphology markers, and [29] they used RAPD marker and morphological marker to study some olive trees. In all previous studies mentioned, there was no co-relation between morphology and genetic data.

The estimates of genetic relationship can be helpful for organizing germplasm, conservation of genetic resources, identification of cultivars for selection of parents for hybridization, and for predicting favorable heterotic combinations. This also helps to reduce the number of samples required for sampling of genetically variable broad range of accessions in breeding programs. Accessions with the most distinct DNA profiles are likely to contain the greatest number of novel alleles, which are likely to uncover the largest number of unique and potentially agronomically useful alleles [27]. However, using both morphological and molecular characterization, provide us with a more complete and informative characterization of the germplasm and this can make breeding programs more easy and useful.

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