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Genetic Diversity Among Sudanese Wheat Cultivars as Revealed by Molecular Markers

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Abstract: Knowledge of genetic diversity in wheat (*Triticum aestivum* L.) cultivars has a considerable impact on the breeding programs. In this study, the genetic relationship of 12 Sudanese wheat cultivars was determined using 184 simple sequence repeats (SSR) and 23 inter simple sequence repeat (ISSR) primer sets. Results indicated that, of the SSRs markers used, 145 (79%) amplified DNA while 39 (21%) did not. A total of 104 of the amplified SSR markers were polymorphic while 41 were monomorphic. All of the 23 ISSR primers amplified DNA and only three of them were monomorphic. The ISSRs produced a total of 146 alleles, of which 47.3% were polymorphic while 52.7% were monomorphic. The similarity coefficients based on SSRs were in the range of 0.37 to 0.61 while those based on ISSRs were in the range of 0.11 to 0.80. Coefficients of similarity generated with SSRs grouped the cultivars into three distinct clusters. In comparison, grouping according to ISSRs have resulted in four clusters, while combined data of both types of markers allowed the grouping of the cultivars into four different clusters. The present study indicates that these markers could be used to obtain fast, accurate and high throughput fingerprinting that revealed the existence of significant variation among the 12 cultivars that can be explore to improve Sudanese wheat.

Key words: *Triticum aestivum* · Clustering · Markers · PcoA

INTRODUCTION

Wheat is a staple food crop worldwide and, in Sudan it is becoming the staple food of most urban populations and many rural areas. In Sudan, wheat production started expanding in the 1960s, when wheat was introduced into the Gezira irrigation scheme as a rotation crop with cotton (*Gossypium* spp. L.) and groundnuts (*Arachis villosulicarpa* L.) [1]. Introduction and screening of wheat genetic materials intensified during the 1980s as a result of increased international collaboration on germplasm improvement. From the mid-1980s, most of the wheat research activities of the Agriculture Research Corporation (ARC) in Sudan were conducted in collaboration with the International Center for Agricultural Research in the Dry Areas (ICARDA) and input from the International Maize and Wheat Improvement Center CIMMYT [2]. In spite of the substantial progress being made in wheat research in Sudan over the past decades through which different promising technology components have been identified, technology verification under farmers' conditions remained quite limited. It has been reported that the weighted average age of varieties in farm's field is 14 years [3]. This lack of use of new cultivars resulted in a narrow genetic base of wheat with limited genetic diversity. Narrow genetic diversity is problematic in breeding for adaptation to biotic and abiotic stresses because there may not be sufficient variation to breed for greater yield under these stressful conditions. Therefore, it is necessary to investigate the genetic diversity in wheat germplasm to understand and in the future broaden the genetic variation available for breeding [4]. Selection of diverse genotypes is a fundamental requirement in wheat breeding programs; this can only be obtained and utilized if gene pool of local and introduced germplasm has sufficient amount of genetic variability [5]. Morphological traits can be used for assessing genetic diversity but are often influenced by the environment. For effective conservation and use of genetic resources, evaluation of genetic variation within collections could be dramatically enhanced by using DNA molecular markers. These DNA markers, when closely linked to genes of interest can be used to select for the desirable allele/s in a marker assisted breeding program [5].

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In recent years, many polymerase chain reaction (PCR) based molecular markers have been extensively applied to estimate genetic variability among wheat cultivars [6,7]. Microsatellite markers or simple sequence repeats (SSR) have proved useful in wheat research since they offer reproducibility, multiallelic nature, co-dominant inheritance, genome specificity, relative abundance and good genome coverage [8,9]. They have been used in the localization of genes to chromosomes [10], identification of quantitative trait loci (QTLs) for yield and quality traits [9], characterization of wheat varieties and germplasm [10,11], MAS and backcrossing [12]. Inter simple sequence repeat or ISSRs [13] have also been widely used for genetic analysis of plants. These markers are arbitrarily primed random markers that also can be exploited for germplasm conservation and to determine the relationships among wheat genotypes [14]. ISSR targets the highly variable microsatellite regions of the nuclear genome providing a large number of polymorphic fragments [15].

The present study was conducted in order to utilize SSR and ISSR markers to estimate genetic diversity among 12 common commercial wheat cultivars commonly grown in Sudan. A long-term goal for this study is to use these data in breeding programs aiming at selecting suitable wheat genotypes for cultivation in Sudan.

MATERIALS AND METHODS

Plant Materials and Protein Analysis: Seeds of twelve elite wheat cultivars (Table 1) were obtained from the Sudanese Agricultural Research Corporation (ARC), Ministry of Science and Technology (MST). These represent almost the complete spectrum of the commercial elite cultivars released during the last 20 years and grown throughout Sudan.

Determination of Genetic Diversity: DNA extraction: Genomic DNA of each cultivar was isolated by a sap-extraction method from 100 mg of fresh tissues. Leaves of 2-week-old seedlings were placed between the two rollers of a sap-extraction apparatus (Ravenel Specialities, Seneca, S.C.) and 1 ml of extraction buffer (50 mM Tris-HCl, 25 mM EDTA, 1 M NaCl, 1% CTAB, 1 mM 1, 10-phenathroline and 0.15% 2-mercaptoethanol) was slowly added to the rollers, immediately mixing with the sap for collection in 1.5-ml microcentrifuge tubes. The extract was incubated at 60°C for 1 h and then mixed with equal volume of chloroform-isoamyl alcohol (24:1). After centrifugation at 12,000 rpm, the supernatant was transferred to a new tube. To precipitate the DNA, isopropanol was added and the contents were incubated for 30 min. The pellet was dried, resuspended in 200 ml of TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) plus 20 µg of RNase and then incubated overnight at room temperature. The DNA solution was mixed with 20 µl of 8 M ammonium acetate and 400 µl of cold absolute ethanol for 30 min, centrifuged for 10 min and air dried at room temperature. The DNA was then resuspended in 200 ml of TE buffer and DNA concentration was quantified by spectrophotometry (TKO100 Fluorometer, Hoefer Scientific Instruments, San Francisco).

PCR Amplification: One hundred eighty-four SSR and 23 ISSR markers were screened for amplification and polymorphism in 12 wheat cultivars. For SSR markers,

Table 1: Wheat cultivars used in the study and their origin, year of release and their pedigrees.

Cultivar	Origin	Year Released	Pedigree/Description
Condor	Australia	1978	Penjamo 62/4 * Gabo 56//Tezanos Pintos Precoz/Nainari60/4/2 * Lerma rojo//Norin 10/Brevor Seln 14/3/3 * Andes. (Recommended for Central Sudan)
Debeira	India	1982	HD 160/5/TOB/CNO67/BB/3/NAI 60*2//TT/SN64/4/HD1954, HD2172. (Recommended for Central Sudan)
Wadi El Neel	Egypt	1987	Chenab 70 / Giza155 (Recommended for Northern Sudan)
El Nielain	Mexico	1990	S948.A1/7*SANTA ELENA, CMH 72A.390-OSDN (Recommended for Northern and central Sudan)
Sasaraib	Mexico (Veery)	1992	KVZ/BUH//KAL/BB, CM 33027-F-15M-500Y-OM-87B-OY-OSDN
Nassr	NA*	1996	W398A/JUP, CM39992-8M-7400M-OAP
Argeen	NA	1998	PAVON/CONDOR
Imam	NA	2000	NA
Tagana	Sudan	2004	NA
Khalifa	Sudan	2004	NA
Bohain	NA	2006	NA
Nebta	NA	2007	NA*NA = Not Available.

the PCR reaction mixture (25 µl total) consisted of 50 mM Kcl and 10 mM Tris-HCl (pH 8.8), 2 mM $MgCl_2$, 125 mM of dNTPs, 50 ng of each primer, 1.0 unit of *Taq* polymerase (Promega) and 20 ng of genomic DNA. Amplification was carried out in a C1000 TouchTM Thermal Cycler (BIO RAD), using a program that consisted of initial denaturation for 3 min. at 94°C, followed by 35 cycles of 1 min. at 94°C, 1 min. at 55°C, 1 min. at 72°C and final extension for 10 min. at 72°C.

For ISSR markers, the PCR amplification was conducted in a 25 ìL volume containing 50 ng of genomic DNA, 50 mM Kcl and 10 mM Tris-HCl (pH 8.8), 1 U Taq DNA polymerase, 1.5 mM $MgCl_2$, 0.25 mM dNTPs and 0.2 ìM primer. The PCR protocol consisted of an initial denaturation at 94ºC for 4 min, followed by 40 cycles of 94ºC for 45 s, annealing at 52ºC for 45 s, 72ºC for 1 min and a final extension step of 72ºC for 10 min.

The amplified PCR products were gel fractionated on 12% polyacrylamide gel (37 acrylamide:1 bis-acrylamide) in a 1X DGGE buffer (40 mM Tris-HCl, 20 mM sodium acetate, 1 mM EDTA). Gels were run for 2.5 hrs in a Hoefer vertical gel apparatus (SE600) at 300 V at 20°C, using a circulating bath temperature set to 20°C. Gels were then stained in ethidium bromide (1 µg/ml) for 10 min, destained in deionized water for 10 min and photographed. Gel images and marker data were processed using Quantity One Software v. 4.0.1 (Bio-Rad Laboratories, Hercules, CA USA).

Data Scoring: Each band was considered as a single locus/allele and was scored as present (1) or absent (0), each of which was treated as an independent character. Genetic diversity analyses were conducted on the basis of the scores.

Statistical Analysis: The scores were used to create a data matrix to analyze genetic relationship using the R software package [16]. A dendrogram was constructed based on Jaccard's dissimilarity coefficient [17] using each marker data for all wheat cultivars following unweighted pair group method (UPGMA) [18]. The combined data for both markers were also utilized for further clustering. The similarity data were used to conduct the principal coordinate analysis (PCoA) and generate the multidimentional scaling [19].

RESULTS

In this study, 184 SSRs and 23 ISSRs markers were used to characterize and evaluate the genetic diversity of 12 wheat cultivars. The SSRs markers were derived from ESTs frequently present in wheat, barley (*Hordeum vulgare* L.), sorghum (*Sorghum bicolor* L.) and maize (*Zea mays* L.). Out of these, 145 (79%) have given clear amplification while 39 (21%) failed to do so. Among the amplified fragments, 104 (71.7%) were polymorphic and 41 (28.3%) were monomorphic. The polymorphic markers produced 773 alleles with PIC values ranging from 0.16 to 0.97. When individual SSRs profiles of the cultivars are considered, the number of polymorphic fragments ranges between 72 in Tagana cv. and 52 in Nebta and Sasaraib. In ISSR analysis, all of the primers amplified the DNA with 20 markers giving clear polymorphism. The markers produced 146 polymorphic alleles. The percentage of marker polymorphism is 47.3 and the number of individual polymorphic fragments ranges between 53 in cultivar Khalifa and 27 in cultivar Tagana. PIC values ranged from 0.16 to 0.99.

Similarity index values obtained from the polymorphic data were used to estimate the genetic relatedness among cultivars. Results shown in Table 2 indicate that

Table 3: Genetic dissimilarity matrix for wheat cultivars as assessed by SSR (lower) and ISSR (upper) markers.

	Tagana	Nebta	WadiElNeel	Sasaraib	Argeen	Debeira	Nassr	Elnielain	Imam	Condor	Bohain	Khalifa
Tagana		0.24	0.25	0.23	0.29	0.20	0.30	0.11	0.22	0.26	0.29	0.23
Nebta	0.44		0.68	0.71	0.67	0.52	0.75	0.57	0.66	0.66	0.69	0.73
WadiElNeel	0.58	0.51		0.63	0.80	0.49	0.70	0.46	0.70	0.64	0.74	0.63
Sasaraib	0.44	0.49	0.39		0.60	0.64	0.64	0.55	0.66	0.67	0.64	0.74
Argeen	0.60	0.40	0.52	0.40		0.60	0.72	0.60	0.69	0.66	0.76	0.71
Debeira	0.56	0.50	0.53	0.50	0.51		0.50	0.58	0.49	0.59	0.43	0.53
Nassr	0.54	0.47	0.54	0.42	0.48	0.50		0.53	0.67	0.68	0.78	0.67
Elnielain	0.48	0.48	0.49	0.40	0.47	0.61	0.49		0.52	0.53	0.46	0.53
Imam	0.48	0.46	0.53	0.44	0.54	0.52	0.49	0.43		0.56	0.65	0.69
Condor	0.55	0.44	0.48	0.42	0.47	0.49	0.51	0.48	0.43		0.66	0.67
Bohain	0.57	0.48	0.48	0.40	0.46	0.42	0.51	0.45	0.48	0.57		0.73
Khalifa	0.53	0.37	0.53	0.39	0.51	0.52	0.55	0.42	0.50	0.52	0.48	

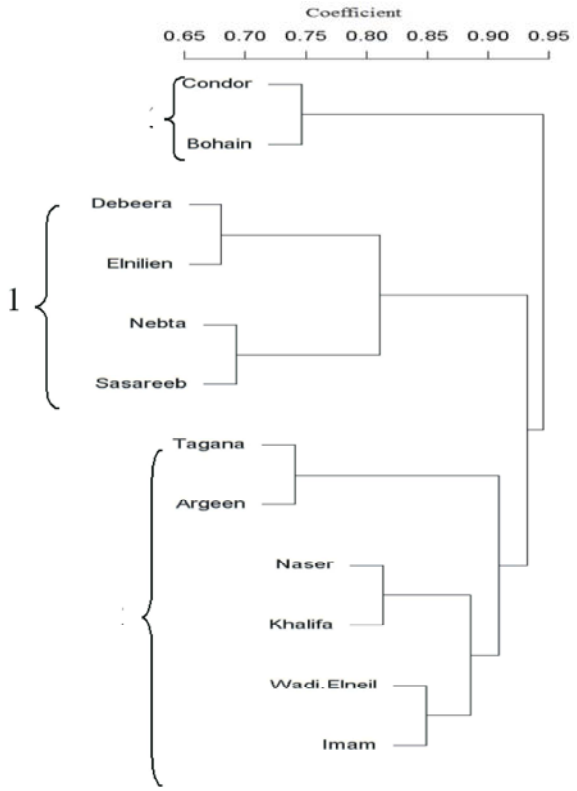

Fig. 1: Dendrogram of 12 wheat cultivars from Sudan based on SSR markers. Values in the *X*-axis correspond to Jaccard's coefficients of dissimilarity.

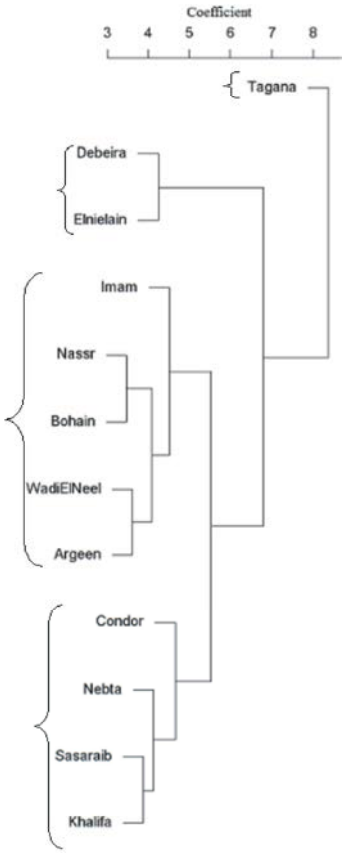

Fig. 2: Dendrogram of 12 wheat cultivars from Sudan based on ISSR markers. Values in the *X*-axis correspond to Jaccard's coefficients of dissimilarity.

the genetic similarity coefficient for all cultivars based on SSR markers ranged from 0.37 to 0.61 with an average of 0.49. It is also revealed that cultivars Elnielain and Debeira are the most closely related genotypes as they showed the highest similarity index (0.61) while the genotypes Nebta and Khalifa are the most distantly related with the lowest index (0.37). A dendrogram based on the similarity values produced from the SSRs was constructed using the UPGMA cluster, in order to illustrate the association between the cultivars used in this study (Fig. 1). The dendrogram revealed three major clusters of wheat cultivars. Cluster 1 contains four cultivars (Debeira, Elnielain, Nebta and Sasaraib) and cluster 3 includes six cultivars (Tagana, Argeen, Nassr, Khalifa, Wadi El Neel and Imam). Cultivars Bohain and Condor are isolated from all other cultivars in a separate group (cluster 2) with similarity coefficient of 0.57. Members of cluster 1 have similarity coefficients ranging from 0.40 to 0.61 while the similarity coefficients for individuals of cluster 3 have ranged from 0.48 to 0.60.

The similarity coefficients based on ISSR markers ranged from 0.11 in cultivars Tagana and Elnielain to 0.80 between Argeen and Wadi El Neel (Table 2). Grouping according to the coefficients of the ISSRs have resulted in four clusters (Fig 2), in which cultivar Tagana occupied a separate cluster (cluster 1). Cluster 2 includes Debeira and Elnielain, cluster 3 contains five cultivars while cluster 4 has four cultivars. It could be noted in this figure that cultivars Condor and Bohain are not in the same cluster as revealed by the SSRs clustering. Although cultivar Argeen has the genetic background of cultivar condor, they are clustered in different groups in the clustering generated from the SSRs or ISSRs data.

Data obtained from both SSR and ISSR were combined and utilized to generate the diversity grouping. Based on the results shown in Fig. 3, the 12 cultivars were grouped into three clusters. Cultivars of cluster 1 are the same ones as in cluster 1 obtained from SSRs clustering and cluster 2 includes cultivar Tagana alone as in

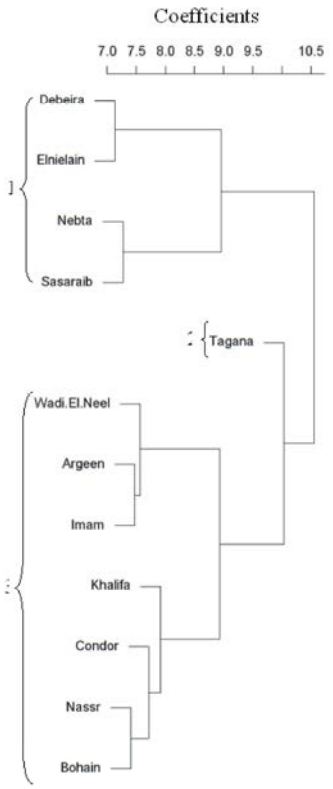

Fig. 3: Dendrogram of 12 wheat cultivars from Sudan based on both SSR and ISSR markers. Values in the *X*-axis correspond to Jaccard's coefficients of dissimilarity.

the ISSRs clustering. In all three dendrograms, cultivars Imam, Argeen and Wadi El Neel are always present in the same group. The same is true for cultivars Khalifa and Nasser. It could also be noted that the recently developed cultivars are represented in each group of all dendrograms.

The association between the cultivars was also examined using the PCoA and the genetic distances were projected into a bidimensional plane. Within cluster 1 in the SSR-based PCoA (Fig. 4), cultivars Sasaraib and Nebta are close to each other while in cluster 2 cultivars Imam and Wadi El Neel are so. Figure 2 also revealed that within the same cluster, members of cluster 2 are more related to each other than members in cluster 1. Generally, Cluster 1 and 2 are close to each other while cluster 3 occupies a marginal coordinate. Cultivars Imam and Wadi El Neel of cluster 2 are the most related to cluster 1. However, the PCoA for data generated from ISSR fingerprinting revealed different grouping pattern (Fig. 5) that did not match the SSR dendrogram clustering. In this figure only three groups are generated in which cultivar Tagana still occupying a separate group, but group 2 and 3 are different than the these groups in Fig. 3. The PCoA of both marker types (Fig. 6) revealed three clusters in which cultivar Tagana is still occupying a separate group, cluster 2 contains two cultivars viz. Debeira and Elnielain while the remaining cultivars aggregate in the third cluster.

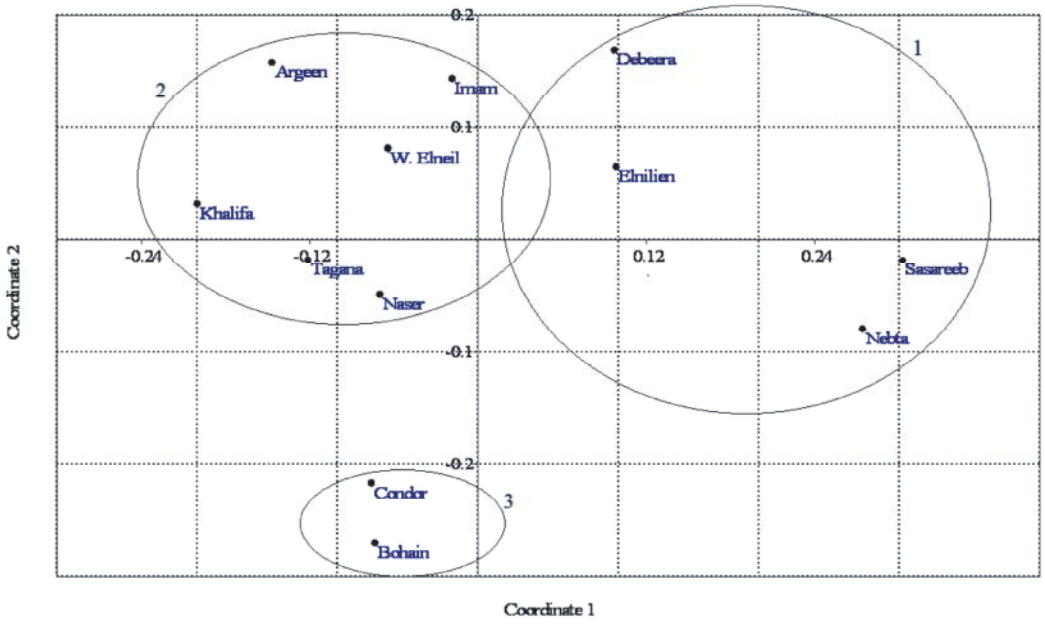

Fig. 4: Principal coordinates analysis obtained by SSR markers

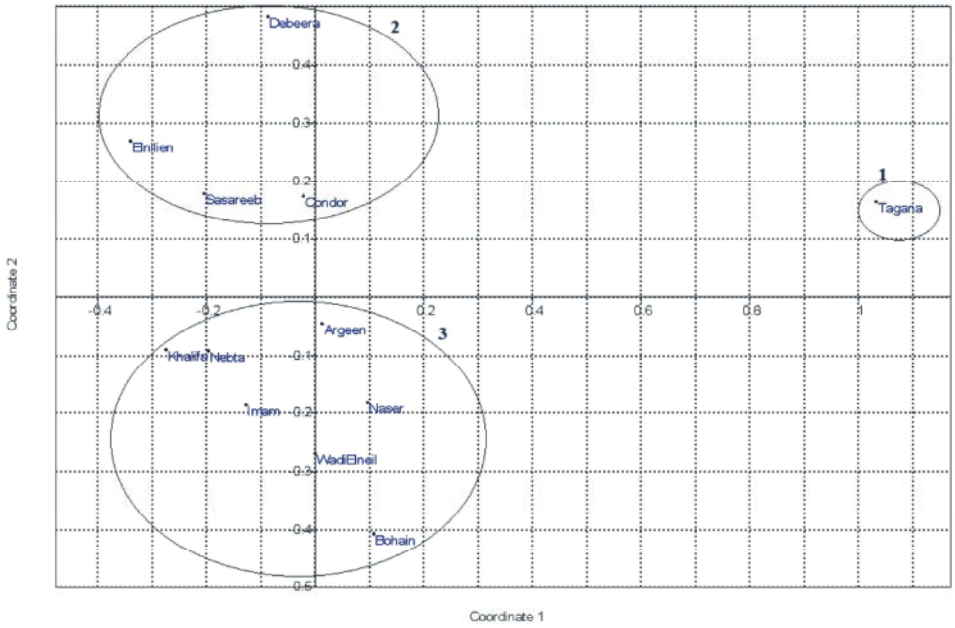

Fig. 5: Principal coordinates analysis obtained by ISSR markers

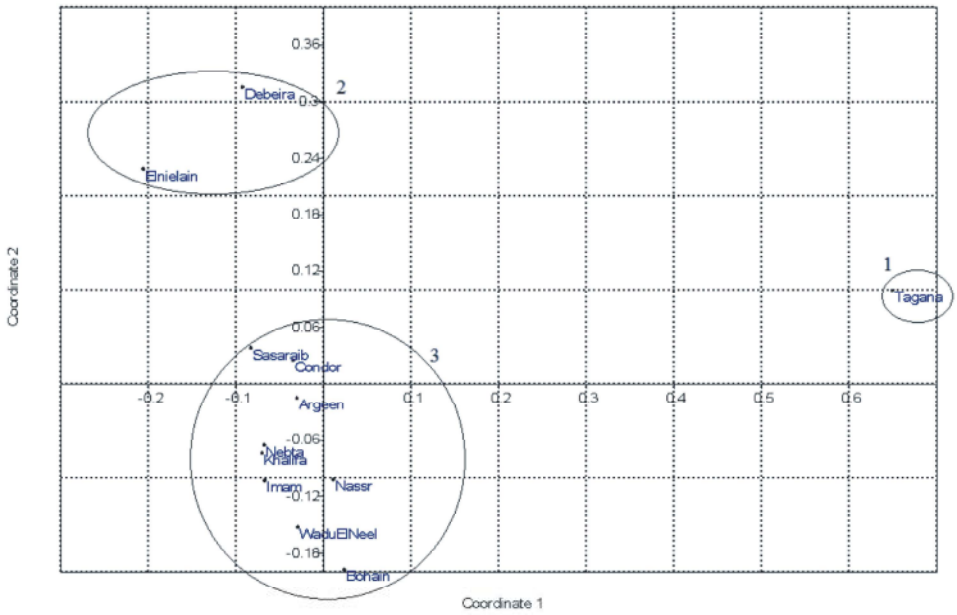

Fig. 6: Principal coordinates analysis obtained by both SSR and ISSR markers

DISCUSSION

Knowledge of genetic diversity among adapted cultivars or elite breeding materials has a considerable impact on the improvement of crop plants. Such knowledge can be obtained from pedigree analysis, morphological traits or using molecular markers [7]. Microsatellites (or SSRs) are powerful molecular markers in wheat because of their high degree of polymorphism [20,21] and also because of their high locus specificity compared to other markers [22]. SSR have been used extensively for designing primer sets which are not only highly polymorphic but also species specific [23]. The cultivars included in this study represent an almost complete spectrum of the bread wheat cultivars released in Sudan during the last 20 years. In this study, 184 SSR primer sets, derived from wheat and its relative cereal crops, were used to detect and estimate genetic

polymorphism in this elite wheat germplasm and to determine the most diverse cultivars for future breeding programs. However, repeated use of germplasm lines with better yield and quality leads to narrowing genetic base of modern cultivars [24].

The dangers of a narrow genetic base of the world's major domesticated food crops have been well documented [25]. According to CYMMIT reports on wheat production in developing countries [1], most of the older Sudanese wheat cultivars are introductions from Australia, Pakistan and India. The recently released varieties such as Wadi El Neel, are Egyptian crosses of Giza-155 (from Egypt) and Chenab-70 (from Pakistan). These reports indicate that during the period 1990-1991, the major varieties grown by farmers were Condor (Australian introduction) and Debeira (an introduction from India) which were grown by 55 and 40% of the farmers, respectively. Heisey and Lantican [3] reported that during the period from 1966 to 1997 the varietal replacement in farmers' filed was very slow due to some constrains. This indicates a low level of improvement practice and consequently a low level of diversity. In the present study, a high polymorphism (71%) was identified with these SSRs indicating their usefulness to screen the diversity level between these cultivars. Comparatively, lower polymorphism has been reported in previous studies using SSR markers in some wheat varieties grown in Egypt [26] and Pakistan [7].

CONCLUSIONS

To the best of our knowledge, we report here for the first time the genetic variation of Sudanese-grown wheat commercial cultivars. Results obtained in this study indicate that most of the cultivars grown in Sudan have high grain protein content. The diversity found among the Sudanese wheat cultivars is very useful for increasing the diversity of the wheat gene pool. All 12 cultivars in this study could be separated based on the ISSR and SSR fingerprints produced by one or more primers. Hence, it could also be concluded that relatively diverse wheat genotypes are used to be grown in Sudan. These findings could have practical uses and may be useful for future studies. The current data can also be used in marker-assisted wheat breeding programs, comparative genetic analysis, for exploiting wheat genetic resources by providing a direct estimate of functional diversity. It could also be useful in enhancement of the breeding efficiency and hence cuts the cost of research studies for genotype identification and genetic diversity studies. It is recommended that diverse varieties with best agronomic traits be used in future breeding programs aiming at creating genetic variability in Sudan's wheat germplasm.

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