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Genetic diversity of rice (*Oryza sativa* L.) accessions collected from Sudan and IRRI using SSR markers

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The assessment of genetic diversity of the traditional rice varieties or landraces is an essential component in germplasm characterization and conservation to identify potential parents. In the present study SSR markers (588 SSR markers) were used for the assessment of genetic diversity and relatedness among 31 rice accessions. These included 18 accessions from Sudan and 13 from IRRI. Among the SSR markers used only 483 generated polymorphic patterns, and showed 1274 alleles. The number of alleles per locus ranged from 2 (about 214 markers) to 5 (RM16820 and AP3206a) with an average of 2.64 alleles per locus. The polymorphic information content (PIC) values ranged from 0.06 (RM3138, RM10671, SKC3, R1M7, R6M30, S07101 and S12041B) to 0.69 (RM7643), with an average of 0.39. The major allele frequency per locus varied from 32% (RM7643) to 97% (RM3138, RM10671, SKC3, R1M7, R6M30, S07101 and S12041B), with an average of 64%. Among the primers used in the present investigation, RM7643 was highly informative as it recorded the highest PIC value (0.69). The UPGMA resulted in allelic richness of four major clusters in which cluster I is composed of a high number of accessions. The pairwise genetic dissimilarity indices revealed the highest genetic dissimilarity of 62.3% between Pipanfary Red1 and FL478. The lowest genetic dissimilarity was found between NBGS3 and NBGS2 (4.1%), but they showed wide dissimilarity with other accessions. The study highlighted the usefulness of the application markers for efficient characterization of the Sudanese rice accessions.

Key words: Rice accessions, genetic diversity, SSR markers, polymorphism.

INTRODUCTION

Information on genetic variability within cultivated crops has a strong impact on plant breeding strategies and conservation of genetic resources (Dean et al., 1999; Simioniuc et al., 2002). This is particularly useful in the characterization of individuals, accessions and cultivars, in determining duplications in germplasm collection and for the choice of parental genotypes in breeding programme (Abu Assar et al., 2005). In Sudan, rice was introduced from Congo since 1905 (Hakim, 1963). It was also known that Oryza *punctata* Kotschy was growing wild for long time in rain-fed depressions (Mac, 1992). Since then, and after numerous introductions and evaluation, rice is now being grown under irrigation in Gezira and White Nile of Sudan and Bahr El-Ghazal, Upper Nile (Malakal), and Jonglei of South Sudan. Although rice has the largest *ex situ* germplasm in the world, which made great contribution to rice breeding (Jackson and Juggan, 1993); however, the genetic diversity of rice in Sudan is not well understood.

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Table 1. Code number, name, type, origin and source of the 18 accessions of rice (*Oryza sativa* L.) native to Sudan and South Sudan and 13 accessions from IRRI.

Name	Type	Source
NBGS1	Landrace	Aweil, Akuem area
NBGS2	Landrace	Aweil, Aryakryak area
NBGS3	Landrace	Aweil, Aulic area
NBGA	Landrace	Aweil, Wotding Achol area
BG400-1	Landrace	Aweil, plot 8
BG90-2	Landrace	Aweil, plot 6
BANBAN	Landrace	Aweil, Madwang area
MASURY1	Cultivated	Kosti, Leya and Hamarya area
PIPANFARY RED1	Landrace	Kosti, Leya and Hamarya area
JAING ARRI RED	Landrace	Kosti, Leya and Hamarya area
SOMMBOY	Landrace	Kosti, Leya andHamarya area
COMARWA	Landrace	Kosti, Leya and Hamarya area
PAINJLA	Landrace	Kosti, Leya and Hamarya area
PIPANFARY	Landrace	Kosti, Leya and Hamarya area
TAGMIZEDO	Landrace	Kosti, Leya and Hamarya area
PIPANFARY RED2	Landrace	Kosti, Leya and Hamarya area
BACTING ARRI	Landrace	Kosti, Leya and Hamarya area
MASURY2	Cultivated	Kosti, Leya and Hamarya area
IR29	Released variety	IRRI gene bank
FL478	Released variety	IRRI gene bank
IR64	Released variety	IRRI gene bank
IR64 - SUB1	Released variety	IRRI gene bank
FR13A	Released variety	IRRI gene bank
IR42	Released variety	IRRI gene bank
KHAO HLAN ON	Released variety	IRRI gene bank
MAZHAN RED	Released variety	IRRI gene bank
DAWE	Released variety	IRRI gene bank
ERATIO	Released variety	IRRI gene bank
DSBRC222 (IRRI 154)	Released variety	IRRI gene bank
IRRI119	Released variety	IRRI gene bank
AZUCENA	Released variety	IRRI gene bank

Exploring diversity in a landrace collection is very important for identifying new genes and further improvement of the germplasm (Thomson et al., 2007). Therefore, detailed study on genetic diversity of the native germplasm of rice in Sudan and South Sudan is very important for the initiation of rice breeding programme that could result in selection of high yielding genotypes under normal and stress conditions. For the assessment of genetic diversity, molecular markers have been found to be generally superior to morphological markers, pedigree, heterosis and biological data (Melchinger et al., 1991). The genetic diversity is commonly assessed by genetic distance or genetic similarity. Among the DNA markers, microsatellites are the most widely used for many purposes such as diversity, genome mapping, varietal identification, determination of the genetic relationship between several sub-species etc. (Ma et al., 2011). The objective of the present study is to assess the extent of correlation and genetic similarity among rice accessions grown in Sudan and South Sudan to be used as parents for future breeding program.

MATERIALS AND METHODS

DNA extraction

A total of 18 rice accessions native to Sudan and South Sudan as well as other 13 genotypes from International Rice Research Institute (IRRI) gene bank were used in this study (Table 1). DNA was extracted from the leaf samples of 14 day old seedlings planted in the green house at IRRI-Philippines using the modified Miniprep Protocol of Thomson et al. (2006). The DNA was quantified using a Thermo Scientific NanoDrop ND -2000/2000C spectrophotometer (Thermo Fisher Scientific, USA). A total of 588 markers were used for the genetic diversity analysis. The motifs for these markers can be found in a public domain (http://www.gramene.org/markers/microsat/).

The polymerase chain reaction (PCR) was carried out using the SSR Programmable Thermal Cycler (MJT55L.scr) as modified by Thomson et al. (2006). The polymerase informative content (PIC) was calculated for each SSR marker as described by Anderson et al. (1993) as follows:

$$PIC_j = 1 - \sum_{i=1}^{n} p_i^2$$

Where, P_{ij} is the frequency of the *j*th allele for the *i*th marker, and

Table 2. SSR markers, Chromosome location, major allele frequency and number of alleles per locus, gene diversity and polymorphism information content (PIC) values among 31 rice (*Oryza sativa* L.) genotypes.

Marker	Chromosome location	Major allele frequency	Number of alleles	Gene diversity	PIC value
RM3138	6	0.97	2	0.06	0.06
RM7643	1	0.32	4	0.74	0.69
RM10671	1	0.97	2	0.06	0.06
RM16820	4	0.44	5	0.71	0.66
AP3206a	1	0.47	5	0.69	0.65
SKC3	1	0.97	2	0.06	0.06
R1M7	1	0.97	2	0.06	0.06
R6M30	6	0.97	2	0.06	0.06
S07101	7	0.97	2	0.06	0.06
S12041B	12	0.97	2	0.06	0.06
*General mean		0.64	2.64	0.46	0.39

*The general mean is the average of 483 polymorphic marker

is summed over *n* alleles.

Allelic diversity of the SSRs was calculated according to the diversity index 'H' as described by Nei (1987) as follows:

$$H_E = \frac{2N}{2N-\mathbf{1}} (1-\sum P^2 i)$$

Where, Pi is the frequency of the i^{th} of k allele.

Genetic similarity between the genotypes was estimated using PowerMarker ver. 3.25 "C. S. Chord, 1967" (Cavalli-Sforza and Edwards, 1967).

Diversity analysis

Based on the DNA fragments, the clearly unambiguous bands were scored visually for their presence and absence with each primer. The scores were obtained in the form of matrix with '1' and '0' which indicates the presence or absence of bands in each accessions, respectively. SSR polymorphisms were measured in terms of major allele frequency, number of alleles per locus, gene diversity and PIC values using PowerMarker software (version 3.25; Liu and Muse, 2005). For the unrooted phylogenetic tree, genetic distance was calculated using the "C.S Chord 1967" distance (Cavalli-Sforza and Edwards, 1967) in PowerMarker with tree viewed using Tree view software.

RESULTS

SSR polymorphism and PIC value

Among the SSR markers used only 483 generated polymorphic patterns, and showed 1274 alleles in the 31 genotypes (Table 2), whereas primers with monomorphic banding patterns were excluded. The number of alleles per locus ranged from 2 (about 214 markers) to 5 (RM16820 and AP3206a), with an average of 2.64 alleles per locus.

SSR markers were highly informative and polymorphic as evident from its PIC value. The PIC values derived from allelic diversity calculated to estimate the informativeness of each primer and allelic frequency among the genotypes were not uniform for all the SSR loci tested. This PIC value ranged from 0.06 (RM3138, RM10671, SKC3, R1M7, R6M30, S07101 and S12041B) to 0.69 (RM7643), with an average of 0.39. The major allele frequency per locus varied from 32% (RM7643) to 97% (RM3138, RM10671, SKC3, R1M7, R6M30, S07101 and S12041B), with an average of 64%. Among the primers used in the present investigation, RM7643 was highly informative since it recorded the highest PIC value of 0.69 followed, respectively by RM149 (0.68), RM10716 (0.68), RM16820 (0.66) and RM23930 (0.65) (Figure 1). Markers that revealed the highest PIC values had highest genetic diversity and lowest allele frequency, whereas markers that showed the lowest PIC value had low genetic diversity and highest allele frequency.

Genetic distance base analysis

The UPAGMA based-dendrogram was obtained from the binary data deduced from the DNA profiles of the samples analyzed where the genotypes that are derivatives of genetically similar types clustered together. The UPGMA resulted in allelic richness of four major clusters observed by rectangular cladogram (Figures 2) with additional sub-clusters within them. Group I is composed of 20 accessions, which can be subdivided into five sub- clusters. Sub-cluster one (GI-1) comprised eight accessions (NBGS3, NBGS2, NBGA, NBGS1, BG90-2, BG400-1, Banban and Masury1) in which NBGS3 showed a narrow genetic similarity with the other accessions (4.10 to 28.42%). Sub-cluster two (GI-2) consisted of two accessions (Pipanfary Red2 and Masury2) with genetic similarity of 15.31%. Whereas Sub-cluster three (GI-3) is composed of five accessions (IR64-Sub1, IR64, IR29, IR42 and FL478) with genetic similarity of 18.37%. Sub-cluster four (GI-4) included two accessions (IRRI119 and DSBRC222) with genetic similarity of 26.84%. On the

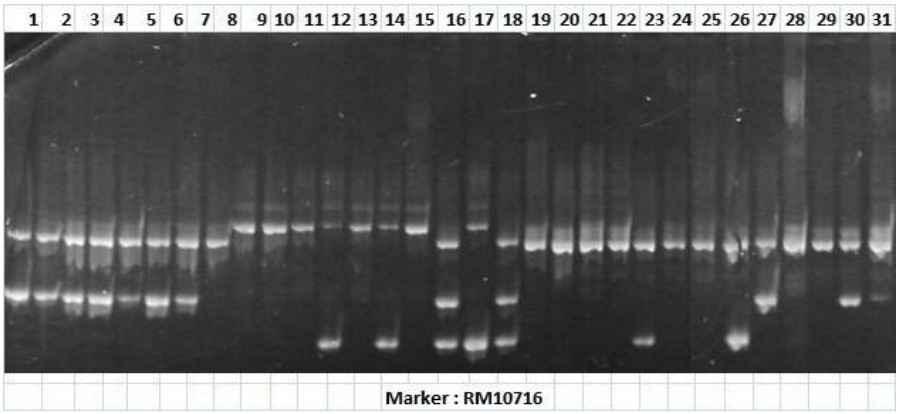

Figure 1. Gel photos polymorphic marker RM10716 showing the bands. Numbers from 1-31 showing the Sudanese and IRRI rice accessions.

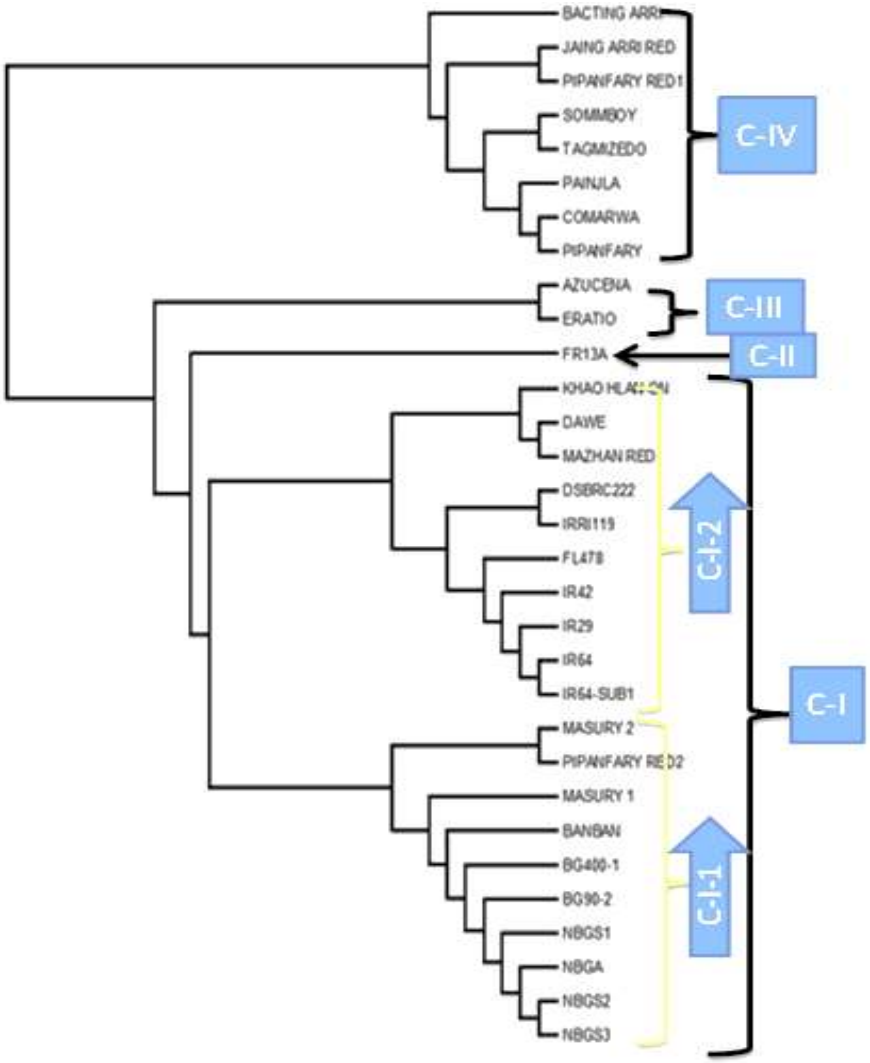

Figure 2. Dendrogram showing a genetic diversity of 31 rice accessions based on polymorphic SSR markers derived from UPGMA cluster using tree view (Win 32).

other hand, sub-cluster five (GI-5) exhibited three accessions (Mazhan Red, Dawe and Khao Hlan On), with a genetic similarity of 26.1%. Group II is composed of only one accession (IR13A), which was distinctly

different from all three accessions (Mazhan Red, Dawe and Khao Hlan On), with a genetic similarity of 26.1%.

Group II composed of only one accession (IR13A), which was distinctly different from all other accessions examined. There were two accessions in GIII (Eratio and Azucena) with genetic similarity of 29.77%. Group IV consisted of eight accessions, which can be subdivided into four subgroups. Subgroup GIV-1 composed of three accessions (Pipanfary, Comarwa and Painjla) which revealed genetic similarity of 10.55 and 12.18%; GIV-2 consisted of two accessions (Tagmizedo and Sommboy) with 11.77% genetic similarity. Subgroup GIV-3 consisted of two accessions (Pipanfary Red1 and Jaing Arri Red) which showed 6.08% genetic similarity, and subgroup GIV-4 composed of only one accession (Bacting Arri).

Pairwise genetic diversity

A dissimilarity matrix was used to determine the level of relatedness among the studied accessions. The genetic similarity (GS) among accessions varied from 0.041 to 0.623 (Table 3). The pairwise genetic dissimilarity indices revealed the highest genetic dissimilarity of 62.3% between Pipanfary Red1 and FL478 and the lowest genetic dissimilarity of 4.1% was found between NBGS3 and NBGS2 (Table 3).

Genetic dissimilarity between the Sudanese rice accessions and IRRI genotypes was comparatively high. Therefore, SSR markers provide an adequate power of resolution to discriminate between the accessions and it could serve as a potential tool in the identification and characterization of genetically distant accessions from different sources.

DISCUSSION

The use of SSR markers to investigate genotypic variations among different genotypes was previously reported by some researchers (Sajib et al., 2012).

The mean alleles of 2.64 per locus detected in the present study was in accordance with the result of Wong et al. (2009) who achieved a value of 2.6 alleles per locus in analysis among 8 Barrio rice cultivars using 12 SSR primers and detecting a total of 31 alleles. Moreover, the result was in the range of 2.0-5.5 alleles per SSR locus for various classes of microsatellites as reported by Cho et al. (2000). However, our results were invariance to those reported by Sajib et al. (2012) and Hossain et al. (2012) who found 3.3, 3.57 and 3.8 alleles per locus, respectively in some rice genotypes. However, the number of alleles 2-5 obtained in this study was slightly lower than the results observed in previous diversity studies in rice genotypes (for example 3 to 9 alleles with an average of 4.53 alleles per locus (Hossain et al., 2007); 3-17 alleles with an average of 7.4 (Yu et al., 2003). Therefore, it could be concluded that the markers with the highest number of discernable alleles are the best markers for molecular characterization and diversity analysis. The variability existing in the number of alleles detected per locus in the present study might be due to the diverse germplasm used and selection of SSR primers with scorable alleles.

The average estimate of gene diversity (*H*) of 0.46 across 31 rice accessions, that was reduced to 0.43 when the analysis was performed with only 18 Sudanese accessions is lower than the estimates of H=0.68 for the rice accessions (*H* = 0.68) reported by Yu et al. (2003) and that of 0.53 reported by Onaga et al. (2013). These results indicate that upland rice accessions grown in Sudan are not sufficiently diverse, although some differences in polymorphism information content (PIC) values were obtained. The SSR marker RM7643 that attained the highest PIC value of 0.69 and high gene diversity of 0.74 was highly informative and can be used for assessing the genetic diversity of rice accessions from Sudan. Hence there was a strong relationship between the PIC value and the number of alleles detected, in which markers that had higher PIC value also had higher number of alleles. This strong positive association between gene diversity of a SSR locus and the number of alleles detected was also reported by Yu et al. (2003) and Onaga et al. (2013). Therefore, confirming that SSRs analysis has a considerable potential for studying the genetic diversity of rice (Xu et al., 2004; Jeung et al., 2005). The level of polymorphism determined by the PIC value (mean = 0.39) in this study is lower than the reported PIC value in previous works (Borba et al., 2009; Upadhyay et al., 2011) who reported an average PIC of 0.6, 0.75 and 0.78, respectively. Evidently, this might be due to the lack of knowledge about diversification of rice accessions in Sudan and it is the first time to use these markers for genetic map of Sudanese rice landraces.

The genetic dissimilarity between the rice accessions was also determined using a dissimilarity matrix. Generally, modern rice cultivars share a relatively narrow genetic background, when compared to the unexplored vast variability existing in rice landraces worldwide. For example, the pedigree of maximum IRRI rice varieties can be traced back to few Indian landraces such as Kitchili Samba, Vellaikar, Tadukan, Thekkan and Eravaipandi (Khush and Virk, 2005). Therefore, it is important not only to conserve landrace genotypes, but also to obtain the gene-pool of rice landraces and unlock valuable genes for breeding purposes (Rabbani et al., 2008).

In the present study, the large range of similarity values for cultivars exhibited by microsatellite markers provides great confidence for the assessments of genetic diversity and relationships, which can be used in future breeding programme. Hence with the aid of microsatellite makers and clustering data, different distantly related rice genotypes may be combined by intercrossing genotypes such as Aweil rice genotypes with IRRI rice genotypes from different clusters to get hybrid varieties with high heterosis. Many studies have also reported significantly greater allelic diversity of microsatellite markers than other molecular markers

Table 3. Pair-wise genetic distance indices among 31 rice accessions obtained from microsatellite marker analysis.

Accessions	Azucena	Bacting Arri	Banban	BG400-1	BG90-2	Comarwa	Dawe	DSBRC222	Eratio	FL478	FR13A	IR29	IR42	IR64	IR64-SUB1	IRRI119
Azucena	0.000															
Bacting Arri	0.524	0.000														
Banban	0.547	0.596	0.000													
BG400-1	0.518	0.588	0.254	0.000												
BG90-2	0.529	**0.618**	0.214	0.208	0.000											
Comarwa	0.566	0.167	**0.616**	0.591	**0.611**	0.000										
Dawe	0.524	0.545	0.315	0.315	0.325	0.575	0.000									
DSBRC222	0.490	0.554	0.350	0.371	0.333	0.566	0.292	0.000								
Eratio	0.298	0.537	0.540	0.503	0.512	0.573	0.493	0.490	0.000							
FL478	0.535	0.566	0.324	0.330	0.300	0.605	0.297	0.290	0.508	0.000						
FR13A	0.505	0.513	0.452	0.441	0.418	0.545	0.413	0.404	0.476	0.378	0.000					
IR29	0.497	0.551	0.284	0.340	0.287	0.590	0.289	0.309	0.511	0.248	0.420	0.000				
IR42	0.504	0.583	0.309	0.348	0.297	0.608	0.301	0.258	0.498	0.308	0.409	0.256	0.000			
IR64	0.482	0.561	0.325	0.366	0.308	**0.611**	0.311	0.276	0.515	0.254	0.424	0.211	0.226	0.000		
IR64-SUB1	0.495	0.549	0.349	0.379	0.329	0.590	0.307	0.286	0.511	0.272	0.391	0.228	0.227	**0.096**	0.000	
IRRI119	0.482	0.568	0.324	0.322	0.307	0.566	0.262	0.268	0.491	0.326	0.399	0.287	0.271	0.292	0.307	0.000
Jaing Arri Red	0.562	0.195	0.609	0.594	**0.611**	0.156	0.602	0.572	0.555	**0.616**	0.554	0.606	0.609	0.595	0.590	0.574
Khao Hlan On	0.492	0.556	0.333	0.334	0.308	0.594	0.302	0.360	0.474	0.310	0.402	0.361	0.294	0.326	0.318	0.346
Masury1	0.530	0.568	0.278	0.208	0.267	0.576	0.347	0.392	0.502	0.358	0.414	0.367	0.366	0.361	0.384	0.357
Masury2	0.506	0.569	0.339	0.353	0.317	0.596	0.333	0.363	0.471	0.314	0.421	0.326	0.334	0.313	0.333	0.371
Mazhan Red	0.510	0.573	0.341	0.353	0.324	0.598	0.261	0.335	0.505	0.300	0.410	0.325	0.292	0.312	0.316	0.300
NBGA	0.528	0.596	0.181	0.175	0.188	0.604	0.305	0.357	0.513	0.296	0.425	0.303	0.354	0.347	0.363	0.324
NBGS1	0.552	0.568	0.207	0.205	0.179	0.594	0.297	0.337	0.522	0.298	0.429	0.290	0.306	0.316	0.340	0.317
NBGS2	0.537	0.590	0.216	0.203	0.202	0.609	0.304	0.381	0.513	0.293	0.421	0.293	0.360	0.335	0.353	0.340
NBGS3	0.530	0.595	0.210	0.191	0.194	0.607	0.301	0.379	0.513	0.297	0.426	0.295	0.363	0.341	0.362	0.336
Painjla	0.570	0.179	**0.615**	0.581	0.604	0.124	0.582	0.560	0.559	0.601	0.563	0.589	0.594	0.585	0.576	0.563
Pipanfary	0.570	0.143	0.599	0.589	**0.616**	0.106	0.567	0.561	0.572	0.587	0.532	0.583	0.606	0.604	0.577	0.568
Pipanfary Red1	0.571	0.205	0.597	0.589	0.601	0.170	0.598	0.585	0.566	**0.623**	0.552	**0.611**	0.607	0.606	0.601	0.584
Pipanfary Red2	0.513	0.568	0.316	0.324	0.308	0.582	0.334	0.351	0.479	0.313	0.426	0.306	0.351	0.325	0.344	0.340
Sommboy	0.568	0.187	0.605	0.601	**0.614**	0.137	0.587	0.592	0.559	0.601	0.540	0.600	**0.622**	**0.618**	0.599	0.580
Tagmizedo	0.561	0.153	0.604	0.578	**0.613**	0.151	0.568	0.569	0.558	0.583	0.531	0.576	0.586	0.597	0.572	0.565

Table 3. Contd.

Accessions	Jaing Arri Red	KhaoHlan On	Masury1	Masury2	Mazhan Red	NBGA	NBGS1	NBGS2	NBGS3	Painjla	Pipanfary	Pipanfary Red1	Pipanfary Red2	Sommboy	Tagmizedo
Jaing Arri Red	0.000														
Khao Hlan On	0.604	0.000													
Masury1	0.562	0.335	0.000												
Masury2	0.607	0.336	0.352	0.000											
Mazhan Red	0.600	0.261	0.369	0.321	0.000										
NBGA	0.600	0.331	0.261	0.331	0.328	0.000									
NBGS1	0.597	0.295	0.281	0.320	0.341	0.168	0.000								
NBGS2	0.607	0.322	0.288	0.326	0.341	**0.098**	0.141	0.000							
NBGS3	**0.622**	0.324	0.284	0.324	0.340	**0.077**	0.145	**0.041**	0.000						
Painjla	0.130	0.606	0.577	0.598	0.605	0.600	0.583	0.608	0.601	0.000					
Pipanfary	0.169	**0.611**	0.588	0.590	0.609	0.598	0.586	0.604	0.602	0.122	0.000				
Pipanfary Red1	**0.061**	0.595	0.567	**0.614**	0.605	0.604	0.587	0.603	0.609	0.140	0.170	0.000			
Pipanfary Red2	0.590	0.360	0.325	0.153	0.329	0.314	0.321	0.325	0.325	0.584	0.573	**0.614**	0.000		
Sommboy	0.132	0.593	0.592	0.581	0.590	0.608	0.586	0.609	**0.613**	0.161	0.145	0.153	0.576	0.000	
Tagmizedo	0.141	0.588	0.577	0.579	0.590	0.599	0.588	**0.610**	0.603	0.124	0.114	0.150	0.570	0.118	0.000

(Sajib et al., 2012; Hoque et al., 2014).

The genetic distance between the 31 rice accessions pairs that ranged from 0.04 to 0.62 indicated a high degree of dissimilarities between the accessions. The high genetic dissimilarity between Aweil accessions and Kosti accessions is an evidence that their source of origin is different from that group of Kosti, and narrow genetic distance among Aweil accessions and also among Kosti accessions may be due to the lack of genetic diversity and they were collected from the same environment. These findings are supported by the report of low genetic diversity for Japanese, Korean, and Venezuelan rice germplasm (Song et al., 2002; Hashimoto et al., 2004; Ghneim et al., 2008). Narrow genetic base of cultivated rice varieties was also reported in other regions, including Latin America (Aguirre et al., 2005) and USA (Xu et al., 2004) and Chile (Becerra et al. 2015). The narrow genetic base observed in this study is not surprising and it could be due to the fact that only a few varieties are available in the country. In addition, several cultivars are named locally and could have arisen through field out-crossing and farmer selection over the years. Thus, one might expect that genetic diversity was on one hand, enhanced by mutation and meiotic recombination, and nonetheless as suggested by Hartl and Clark (1997) curtailed by genetic drift and natural and artificial selection.

The four main clusters of UPGMA analysis in the 31 rice accessions of the present study is unlike the cluster grouping of Sajib et al. (2012) who found five clusters for aromatic landraces, and that of Onaga et al. (2013) who found three main clusters for IRRI and Ugandan rice cultivars, and Prabakaran et al. (2010) who reported six clusters for other rice landraces. The wide variability existed in this study, between the thirty-one genotypes as revealed by the microsatellite markers provides greater confidence for the assessment of genetic diversity and relationships, which may be useful in marker-assisted selection in breeding programme.

From the results it could be concluded that the DNA fingerprinting and genetic diversity of Sudanese rice accessions using SSR markers is effective. The information about the genetic diversity will be useful for proper identification and selection of appropriate parents for breeding programme including gene mapping, and ultimately for emphasizing the

importance of marker assisted selection (MAS) in Sudanese rice improvement.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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