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Assessment of Genetic Diversity Using SSR Markers in Some Rice Genotypes

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Abstract: A study was conducted at the Institute of Plant Breeding, Seed Science and Population Genetics, University of Hohenhiem, Stuttgart, Germany, during 2008 to assess genetic diversity among 17 rice genotypes by using 36 SSR markers. All the primers showed polymorphism. A total of 320 alleles was detected at the markers loci across the rice genotypes. The mean number of alleles per locus was 8.8. Cluster analysis grouped the rice genotypes into 5 major groups as the genotypes that are derivatives of a genetically similar type clustered more together. These genotypes represented a valuable source of diversity which can be used in the breeding programmes.

Key words: Rice; genetic diversity; SSR markers

INTRODUCTION

Rice, *Oryza sativa* L., is the world's second most important cereal crop, grain production of rice being exceeded only by that of wheat. Rice is the major caloric source, with nearly 2.5 billion people depending on it as their main food (FAO 2004). Rice has been grown in Sudan since 1905, but on a very limited acreage and information about methods of production is lacking (Farah 1981). Swamp and Upland varieties were first tried at the Gezira Research Farm in 1951. Although rice cultivation in the Sudan was known for some time, especially in Southern Sudan and White Nile areas, large–scale production started only in the 1950s in Malakal (Upper Nile Province), and in 1960 in Aweel (Bahr El Gazal Province). Rice research in the Sudan was carried out mainly by external

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expertise and focused on identification of suitable varieties and cultural practices, like sowing date, irrigation and fertilizers.

Rice breeders are interested in selecting high yielding genotypes with improved yield and other desirable agronomic characters. Molecular marker technologies can assist conventional breeding efforts and are valuable tools for the analysis of genetic relatedness and identification and selection of desirable genotypes for crosses as well as for germplasm conservation in gene banks, prediction of hybrid performance, heterosis and tagging of valuable quantitative trait loci (QTL) and genes (Xiao *et al.* 1996; Zou *et al.* 2000). Molecular markers, such as SSRs, have been widely used in rice germplasm evaluation. The use of SSRs to interpret population structure provides much greater resolution than other types of markers (RFLPs), because of high level of polymorphism at SSR loci (Cho *et al*. 2000). In rice, the highly polymorphic nature of SSR motifs is coupled with a low level of homoplasy observed in *O.sativa* cultivars (Chen *et al*. 2002), providing an appropriate tool for population genetic studies. About 2240 microsatellite markers are now available through the published high-density linkage map (McCouch *et al.* 2002) or public database.
This study was aimed to investigate genetic diversity and relationships among 17 rice genotypes by using microsatellite markers so that diverse genotypes could be selected for breeding purposes.

MATERIALS AND METHODS

Plant material

The plant material used in this study comprised 17 rice genotypes, viz 7 NERICA genotypes from WARDA (West African Rice Development Association, Benin), 8 genotypes from IRRI (International Rice Research Institute, Philippines) and 2 local genotypes (Table 1). The material was kindly provided by Ahmed Mohamed Mustafa, National Coordinator, Rice Research programme, Agricultural Research Corporation (ARC), Sudan. Seeds were sown in 5 rows in decomposite soil potted in plastic trays (0.5x0.3 m length), which were placed in the growth chamber to raise seedlings.

Table 1. Rice genotypes used in microsatellite markers diversity study

Genotype	Origin	Genotype	Origin
NERICA 2	WARDA	YUNLU 30	China
NERICA 4	WARDA	YUNLU 33	China
NERICA 5	WARDA	YUNLU 33	China
NERICA 12	WARDA	BALADI	Local
NERICA 14	WARDA	MASRI	Local
NERICA 15	WARDA	WAB-1-38-19-14-P2-HB	IRRI
NERICA 17	WARDA	WAB880-1-38-19-8	IRRI
YUNLU 22	China	WAB891SG12	IRRI
YUNLU 26	China		

WARDA= West African Rice Development Association, IRRI= International Rice Research Institute.

DNA extraction

DNA was extracted from 3 week old seedlings, using the CTAB protocol as described by Saghai-Maroof *et al*. (1984). The leaf samples were taken from 5 seedlings per accession. The quality and quantity of the extracted DNA was checked on 3% agarose gel, and working dilutions were prepared for PCR reactions. A total of 36 SSR primers (Table 2) were chosen to cover all rice chromosomes, on the basis of previous studies.

Polymerase chain reaction (PCR)

The PCR was conducted in a final volume of 10µl reaction mix, containing 10ng/10µl of template DNA, 4.3µl water bidest, 1.5 Mm $MgCl_2$, 1x1.5m M Mg Cl_2 of 10×pcr-Puffer, 0.2mM per dNTP-Mix, 0.5 U/10µl of Tag Polymerase, 250 nM of Forward primer and 250 nM of Reverse primer. The amplification of template DNA was carried out in a PTC- 100^{TM} programmable thermal cycler. The reaction was started with a denaturation step at 94°C for 3 min, followed by primer annealing at 55°C for 1 min and elongation at 72°C for1 min. After 39 cycles, the reaction was kept at 72°C for 10 min for the final extension. The annealing temperature was adjusted, according to the specific requirements of each primer combination. The PCR products were separated electrophoretically in 3% metaphor agarose gel at 140 v for 2.15 h. The gels were stained in Ethidium Bromide (5 µg/ml) for 5 min, de-stained for 15 min and then examined under ultraviolet light, using a video capture system (Flowgen IS 1000).

Table 2. SSRs markers used, their sequence, fragment sizes and chromosome location

Marker	Forward sequence	Reverse sequence	Tm (°C)	Fragment size(bp)	Chromosome no.
RM421	AGCTCAGGTGAAACATCCAC	ATCCAGAATCCATTGACCCC	49	220-272	5
RM204	GTGACTGACTTGGTCATAGGG	GCTAGCCATGCTCTCGTACC	48	108-181	6
RM72	CCGGCGATAAAACAATGAG	GCATCGGTCCTAACTAAGGG	55	154-235	8
RM222	CTTAAATGGGCCACATGCG	CAAAGCTTCCGGCCAAAAG	54	209-280	10
RM220	GGAAGGTAACTGTTTCCAAC	GAAATGCTTCCCACATGTCT	45	100-133	1
RM539	GAGCGTCCTTGTTAAAACCG	AGTAGGGTATCACGCATCCG	52	225-316	6
RM303	GCATGGCCAAATATTAAAGG	GGTTGGAAATAGAAGTTCGGT	66	154-218	4
RM114	CAGGGACGAATCGTCGCCGGAG	TTGGCCCCCTTGAGGTTGTCGG	66	343-414	3
RM249	GGCGTAAAGGTTTTGCATGT	ATGATGCCATGAAGGTCAGC	55	100-134	4
RM25	GGAAAGAATGATCTTTTCATGG	CTACCATCAAAACCAATGTTC	51	128-154	8
RM3	ACACTGTAGCGGCCACTG	CCTCCACTGCTCCACATCTT	51	109-142	6
RM52	CTACTCGCGCGTGGAGTT	TGTCTTACTGGTGAAGCTGG	49	163-187	8
RM229	CACTCACACGAACGACTGAC	CGCAGGTTCTTGTGAAATGTT	48	106-133	11
RM547	GGCGAATTCTTTGCACTTGG	ACGGTTTGGTAGGGTGTCAC	55	150-213	5
RM224	ATCGATCGATCTTCACGAGG	TGCTATAAAAGGCATTCGGG	52	117-160	11
RM7	TTCGCCATGAAGTCTTCTCG	CCTCCCATCATTTCGTTGTT	51	143-180	3
RM257	CAGTTCCGAGCAAGAGTACTC	GGATCGGACGTGGCATATG	48	170-226	9
RM585	CAGTCTTGCTCCGTTTGTTG	CTGTGACTGACTTGGTCATAGG	49	174-243	6
RM243	GATCTGCAGACTGCAGTTGC	AGCTGCAACGATGTTGTCC	51	111-133	1
RM254	AGCCCCGAATAAATCCACCT	CTGGAGGAGCATTTGGTAGC	54	150-178	11
RM234	ACAGTATCCAAGGCCCTGG	CACGTGAGACAAAGACGGAG	51	129-178	7

Table 2 contin.

Marker	Forward sequence	Reverse sequence	Tm (°C)	Fragment size(bp)	Chromosome no.
RM225	TGCCCATATGGTCTGGATG	GAAAGTGGATCAGGAAGGC	51	128-200	5
RM286	GGCTTCATCTTTGGCGAC	CCGGATTCACGAGATAAACTC	51	96-145	11
RM5	TGCAACTTCTAGCTGCTCGA	GCATCCGATCTTGATGGG	51	103-115	1
RM259	TGGAGTTTGAGAGGAGGG	CTTGTTGCATGGTGCCATGT	47	150-173	1
RM154	ACCCTCTCCGCCTCGCCTCCTC	CTCCTTCCTCCTGCGACCGCTCC	66	166-205	2
RM21	ACAGTATTCCGTAGGCACGG	GCTCCATGAGGGTGGTAGAG	52	129-185	11
RM235	AGAAGCTAGGGCTAACGAAC	TCACCTGGTCAGCCTCTTTC	52	86-110	12
RM338	CACAGGAGCAGGAGAAGAGC	GGCAAACCGATCACTCAGTC	51	169-200	3
RM167	GATCCAGCGTGAGGAACACGT	AGTCCGACCACAAGGTGCGTTGTC	56	122-176	11
RM13	TCCAACATGGCAAGAGAGAG	GGTGGCATTCGATTCCAG	50	124-163	5
RM168	TGCTGCTTGCCTGCTTCCTTT	GAAACGAATCAATCCACGGC	59	92-117	3
RM333	GTACGACTACGAGTGTCACCAA	GTCTTCGCGATCACTCGC	51	170-216	10
RM226	AGCTAAGGTCTGGGAGAAACC	AAGTAGGATGGGGCACAAGCTC	51	182-345	1
RM20A	ATCTTGTCCCTGCAGGTCAT	GAAACAGAGGCACATTTCATTG	52	208-344	12
RM210	TCACATTCGGTGGCATTG	CGAGGATGGTTGTTCACTTG	50	106-189	8

Statistical analysis

The fragment sizes were assessed, using the software package Total Lab (Phoretix, Newcastle, UK). The resulting SSR data were analysed statistically, using the software "Tool for Population Genetic Analysis" (TFPGA) version 1.3 (Miller 1997), to calculate the distance matrix between genotypes based on the modified Roger distance (MRD) measure. Cluster analyses (CA) were performed for construction of dendrograms, based on the unweighed pair group method with arithmetic mean (UPGMA) procedure, using the software package "Molecular Evolutionary Genetics Analysis" MEGA 4, (Tamura *et al*. 2007). The genotypes were grouped using the neighbour joining method with MEGA 4.

Genetic diversity of rice genotypes was measured according to Nei's average gene diversity, using the "Population Genetic Analysis" (PopGene) software version 1.31 (Yeh *et al*. 1999).

The polymorphism at each SSR locus was measured in terms of number of alleles per locus and observed heterozygosity (Ho), using the "Population Genetic Analysis" (PopGene) software version 1.31 (Yeh *et al*. 1999). The polymorphic information content (PIC), which is an estimation of discriminatory power of a SSR marker locus, was calculated according to the formula: PIC= 1-(∑pi2), where i is the total number of alleles detected for a SSR marker, and pi is the frequency of the i th plus allele in the set of the 17 rice genotypes investigated (Weir 1996).

RESULTS AND DISCUSSION

Polymorphism of SSR markers

In the present investigation, microsatellite markers were used to characterize and to assess genetic diversity among 17 rice genotypes. All the 36 RM primer showed polymorphism among the genotypes. A total of 320 alleles were detected at 36 microsatellite markers across 17 rice genotypes. The number of alleles per marker ranged from 7 (RM 25, RM 235, RM 13 and RM 333) to 11 (RM 20 A and RM 210), with an average of 8.8 alleles (Table 3).This indicates a greater magnitude of diversity among the genotypes assessed. Many studies have also reported

significantly greater allelic diversity of SSR markers than other molecular markers (McCouch *et al*. 2001). The number of detected alleles per marker was higher than that reported in a previous work by Alvarez (2007).

The PIC varied from a minimum of 0.72 (RM 235) to a maximum of 0.90 (RM 21 and RM 20A), with an average of 0.84 (Table 3). Marker RM 20A showed the highest allelic diversity and PIC value. The markers showed an average PIC value of 0.84, which confirms that SSR markers used in this study were highly informative. Markers with PIC values of 0.5 or higher are highly informative for genetic studies and are extremely useful in distinguishing the polymorphism rate of a marker at a specific locus (De Woody *et al*. 1995). The genotypes used in the present study were more diverse due to differences in origin, ecotype and speciation. Microsatellite markers exhibited high PIC values because of their codominant hyper variable, abundant and well distributed throughout the rice genome (Temnykh *et al*. 2001).

Genetic diversity

Number of alleles detected in individual genotype ranged from 1.6 (YUNLU 30) to 2.3 (NERICA 2), with an average of 1.7 (Table 4). The majority of rice genotypes showed a single allele (the rest of the genotypes showed two alleles). Genetic heterogeneity is a common feature of rice accessions, despite the high inbred nature of the species (Jain *et al*. 2004). Most of the accessions were derived from pedigree breeding programmes. Pedigree selection involves homozygous breeding; this should fix the genetic material revealing no more than one allele per locus. Although rice is a self pollinating species, previous studies have reported more than one allele for a given single locus marker in a single cultivar. Similar results have been reported for some self pollinated crop species, e.g. wheat (Roder *et al*. 2002) and barley (Sjakste *et al*. 2003).

Table 3. Diversity of the 36 SSR markers among the 17 rice genotypes

Marker	Total no. of observed alleles	PIC	Marker	Total no. of observed alleles	PIC
RM421	8	0.74	RM243	9	0.87
RM204	9	0.86	RM254	9	0.88
RM72	9	0.87	RM234	9	0.88
RM222	10	0.88	RM225	9	0.86
RM220	10	0.86	RM286	10	0.87
RM539	8	0.83	RM5	9	0.83
RM303	9	0.88	RM259	9	0.88
RM114	9	0.89	RM154	9	0.79
RM249	9	0.85	RM21	10	0.90
RM25	7	0.81	RM235	7	0.72
RM3	9	0.85	RM338	9	0.83
RM52	9	0.87	RM167	8	0.81
RM229	9	0.81	RM13	7	0.80
RM547	9	0.88	RM168	8	0.83
RM224	9	0.88	RM333	7	0.84
RM7	8	0.84	RM226	10	0.88
RM257	9	0.87	RM20A	11	0.90
RM585	9	0.85	RM210	11	0.88
Mean				8.8	0.84
St.Dev				0.9	0.03

PIC= Polymorphic Information Content.

The percentage of polymorphic loci of markers was generally higher for NERICA's genotypes, ranging between 52.78 (for YUNLU 30 and WAB 891SG 12) and 80.56(for NERICA'S 2, 14 and 15), with an average of 68.30 (Table 4).

Nei's average gene diversity ranged from 0.2397 to 0.4310 with an average of 0.3449 (Table 4). The majority of genotypes exhibited Nei's average gene diversity of 0.3. NERICA 14 showed the highest gene diversity of 0.4, and this may be due to its origin (interspecific hybridization between *O.sativa and O. glaberima*). In comparison, a published survey of 234 global rice accessions had overall gene

diversity of 0.70, an Indica diversity of 0.55 and a tropic Japonica gene diversity of 0.47 (Garris *et al*. 2005).

These results are higher in comparison with our results, and this may be due to the large number and wider diversity of accessions used in that study. Gao *et al*. (2005) reported a gene diversity of 0.68 in Indica group and 0.57 gene diversity in Japonica group.

Table 4. Genetic diversity of 17 rice genotypes detected using SSR markers

Genotype	Average no. of alleles	Percentage of polymorphic loci (0.05)	Nei's average gene diversity
NERICA 2	2.3	80.56	0.4310
NERICA 4	1.8	66.67	0.3083
NERICA 5	1.7	63.89	0.2589
NERICA 12	1.7	66.67	0.2939
NERICA 14	2.2	80.56	0.4021
NERICA 15	2.0	80.56	0.3715
NERICA 17	1.8	72.22	0.3072
YUNLU 30	1.6	52.78	0.2397
YUNLU 33	1.8	72.22	0.3394
YUNLU 34	1.8	69.44	0.3669
BALADI	1.9	66.67	0.3342
MASRI	1.9	66.67	0.3299
WAB880-1-38-19-8	1.9	61.11	0.3090
WAB891SG12	1.9	52.78	0.2809
WAB-1-38-19-14-P2-HB	2.0	69.44	0.3578
YUNLU 22	1.9	66.67	0.3254
YUNLU 26	2.0	72.22	0.3672
Mean	1.7	68.30	0.3449
St. Dev.	0.8387		0.2421

Cluster analysis

Cluster analysis was used to group the genotypes according to the constructed dendrogram. The dendrogram revealed that the genotypes that are derivatives of genetically similar types clustered more together. The genotypes clustered in five major groups (Figure1). Cluster 1 was the largest one, consisting of 6 of NERICA's genotype. NERICAs in group 1 were further divided into two subgroups, with NERICA 2, 4 and 5 forming the first subgroup and others (NERICA 12, 14 and 15) the second subgroup, confirming the close genetic relationship for these genotypes. Cluster 2 comprised only one genotype (NERICA 17). This result agrees with the results obtained by Semagn *et al*. (2006), who studied genetic relationship among 18 NERICA genotypes. They found distinct separation of NERICA's 1 to 7 from NERICA's 8 to 18 in both clusters. The highest genetic distance was found between NERICA 6 and 17, which is as expected as both were derived from different Japonica genotypes. YUNLU's genotypes clustered in two separate groups 3 and 5, while cluster 4 consisted of only local genotypes. WAB's genotypes plus YUNLU 22 and 26 were clustered in a separate group. This may be due to the closeness of their genetic origin (japonica subspecies), however, YUNLU 22 and 26 are very similar in morphological and agronomic traits and they were different from other YUNLU genotypes. Brondani *et al*. (2006) reported six clusters constructed from analysis of 192 rice accessions. Ram *et al*. (2007) reported that the cluster dendrogram revealed 5 clusters from 35 rice accessions.

CONCLUSION

The allelic diversity revealed by the 36 SSR primers was sufficient enough to distinguish between the assessed genotypes. The grouping of genotypes, on SSR polymorphism data, corresponds well to their origin.

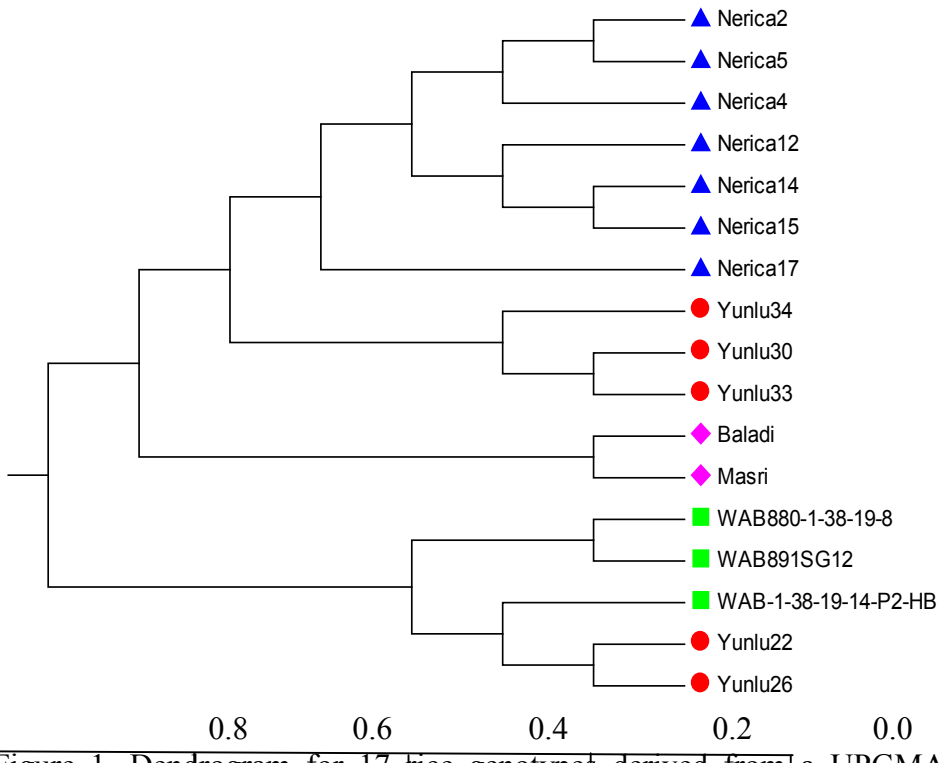

Figure 1. Dendrogram for 17 rice genotypes derived from a UPGMA cluster analysis based on 36 SSRs markers

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تقويم التنوع الوراثي فى بعض سلالات الأرز باستخدام بادئات تكرار تسلسل بسيط

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المستخلص: أجريت دراسة بمعهد تربية النبات وعلوم البذور ووراثة العشائر ، جامعة هوننهايم ، شتوتقارت – ألمانيا خلال عام 2008 لتقويم التنوع الوراثي لسبعة عشر طرازاً وراثياً للأرز بإستخدام 36 بادئة تكرار تسلسل بسيط . أظهرت جميع البادئات تعدداً فى الأشكال . أمكن التعرف على 320 اليل لكل المواقع الوراثية لطرز الأرز ، وكان متوسط عدد الألـيلات فـي الموقـع الـوراثي 8.8 . أظهـر التحليـل العنقـودى خمـس مجموعات رئيسة ، حيث أن الطرز الوراثية المتشابهة وراثياً تتجمع مع بعضها البعض . مثلت هذه الطرز مصدراً مهماً للتباين يمكن الإستفادة منه في برامج التربية .

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