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Molecular Characterization and Genetic Diversity Analysis of Elite African Lowland Rice Varieties using SSR Marker System

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Abstract: *The genetic diversity and phylogentic relationship of six improved African lowland rice varieties, namely BW-348-1, FARO-44, FARO-57, NERICA-L-19, NERICA-L-34 and WITA-4 was investigated using 129 SSR primers on the twelve chromosomes of rice. DNA was extracted by modified cetyl trimethyl ammonium bromide (CTAB-A) method. The banding pattern was recorded in the form of 0-1 data sheet which was analyzed using unweighted pair group method with arithmetic mean (UPGMA) based on Jaccard's similarity coefficient. The result reveals that six varieties produced a total of 492 alleles and the average number of alleles per locus was 3.8. The polymorphism Information Content (PIC) values ranges from 0.0 to 0.375 and gene diversity ranges from 0.0 to 4.4. Sixty-two (62.79%) of the primers revealed at least 3 alleles while 37.21% produced monomorphic bands for the six varieties. The size of the detected alleles produced from the SSR primer sets ranged from 50bp to 400bp, showing a large difference in the number of repeats between the different alleles. The SSR base dendogram generated and the Principal Component Analysis (PCA) clustered two genotypes BW-348-1 and FARO-44 together and three genotypes FARO-57, NERICA-L-19 and WITA-4 together. Though WITA-4 branched separately from the other two, NERICA-L-34 occupied a distinct position that is different from the other genotypes.*

Keywords: *Genetic diversity, simple sequence repeats (SSR) marker, dendrogram.*

1. INTRODUCTION

Rice is the principal food of nearly half of the world's people and more than 90% of the crop is grown in developing countries, where food supply is an acute problem. Much success has been gained in rice production over the past 35 years as it has more than doubled from 257 million tonnes to 596 million tonnes in 1999 (Khush *et al.,* 2001). This increase can be attributed to the large-scale adoption of improved rice varieties and technology. (Fagade, 2000; Falusi, 1997) reported that rice production In Africa in the recent years (2001-2005) has been expanding at the rate of 6% per annum, but much of the increases in production are rather attributed to land expansion than increases in productivity. Since the development of interspecific high yielding rice varieties by Africa rice centre, new gene pools has been opened and increased biodiversity made available for world of science (Somado et al., 2008). Genetic diversity among individuals reflects the presence of different alleles in the gene pool, and hence, different genotypes within populations. Genetic diversity analysis provides vital and powerful data that help in the understanding of genetic variation and improved conservation strategies. Diverse data sets obtained from the study of morphology (Bourgoin *et al.,* 1995), physiology (Morishima *et al.,* 1997), isozymes (Hamrick and Godt, 1997; Farooq and Azam, 2002) and storage protein profile (Smith *et al.,* 1987) have been used to assess genetic diversity. Rahman *et al.* (2011) reported that molecular technique provides a more reliable set of data in the study of genetic diversity of rice than the ones provided by morphological and physiological methods. Since Botstein *et al.,* (1980) developed the first molecular marker i.e Restriction fragment length polymorphism (RFLP), other marker systems like random amplified polymorphic DNA (RAPD), Amplified fragment Length Polymorphism (AFLP), simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) have been used in germplasm identification, assessment of genetic diversity and determination of phylogentic relationships amongs various crop genotypes. Simple sequence repeats (SSR), also known as microsatellites or sequence-tagged microsatellite sites (STMS), are simple tandemly repeated, di- to tetra-nucleotide sequence motifs such as (CA)n, (AAT)n and (GATA)n flanked by

unique sequences and they occur frequently throughout plant genomes (Tautz and Renz, 1984). Microsatellites have a particular attribute in that they suffer higher rates of mutation than the rest of the genome and this can be said to be responsible for their wide use as valuable markers for genetic diversity analysis. Also they detect allelic variation very easily (Jarne and Lagoda, 1996). Mc Couch *et al.,* (2002) reported that SSR markers have been widely used in molecular characterization and genetic diversity analysis of aromatic landraces of rice (Sajib *et al.,* 2012), to analyze genetic structure within the cultivated rice (Garris *et al.,* 2005), to evaluate genetic diversity among strains of wild rice (Shishido *et al.,* 2006) and among cultivars of cultivated rice (Yu *et al.,* 2003; Jain *et al.,* 2004; Zeng *et al.,* 2004; Jayamani *et al.,* 2007).

Development of high-yielding varieties can be achieved through genetic resources identification and characterization of genotypes and varieties with desirable traits. The existing diversity in plant populations and varieties is used to produce new varieties and to improve on the existing varieties (Guimara˜es, 2009). Several reports from various rice breeding programs across the world have indicated narrow genetic diversity in the varieties developed and released to farmers (Cuevas-Pe´rez *et al.,* 1992) and (Montalban *et al.,* 1998). Furthermore Langridge and Chalmer (2004) noted in their study that the primary gene pools of many crop plants are so depleted in genetic variability that breeders are now exploring the potentials of wild relatives for sources of disease resistance and other traits. But the utilisation of these wild relatives is greatly hindered by hybridisation barriers preventing interspecific crosses and/or by undesirable characteristics inherent in exotic germplasm. Therefore this limitation has made breeders to utilize exotic germplasm as source of genes for disease and insect resistance and have relied on repeated intercrossing of adapted elite genotypes for improvement of quantitative traits, like yield, and qualitative traits (Langridge and Chalmer 2004). In the present study, six elite varieties of Africa rice were analyzed for genetic variation using SSR markers. The objective of the study was to use DNA fingerprinting and genetic diversity analysis of elite varieties of Africa rice to measure the extent of genotypic differences and genetic relationship and to determine the possibility of crossing elite genotypes to create new lines for rice breeding programs.

2. MATERIALS AND METHODS

2.1. Germplasm Collection and Genomic DNA Extraction

A total of 6 rice genotypes were evaluated in this study. The genotypes are BW-348-1, FARO-44, FARO-57, NERICA-L-19, NERICA-L-34 and WITA-4. All the seeds were collected from the Genetic Resource Center (GRC), Africa Rice Center, Ibadan, Nigeria and germinated under aseptic condition by keeping them at 30ºC for 1 day and then raised in pots in a net house. At 3 weeks after germination, leaves, about 2cm long, from each plant was harvested and bulked for each genotype. Total genomic DNA isolation was carried out using modified CTAB-A method based on the classical Doyle and Doyle (1987) protocol.

The quality of DNA was also checked by DNA quantification using a Thermo Scientific NanoDrop™ 1000 spectrophotometer (Thermo Fisher Scientific, USA).

2.2. SSR Markers and PCR Amplification

A total of one hundred and twenty nine SSR primer pairs were selected at random for the genetic diversity analysis of the six elite Africa rice varieties. Primers that showed polymorphic banding patterns were selected whereas primers that showed monomorphic banding patterns were excluded. Finally, 9 microsatellite primers with a distinct chromosome number were used for final polymerase chain reaction (PCR) amplification. Prior to DNA amplification, a PCR cocktail was prepared containing all required components. PCR amplification reactions were done in 10 µl reaction mixtures containing 3 µl of diluted template DNA, 0.5 µl of each forward and reverse primer, 0.25 µl of 10 mM dNTPs, 1.5 µl of 10x buffer, 0.2 µl of *Taq* polymerase, 1.8 µl of MgCl2 and 2.25 µl of ddH2O. A DNA thermal cycler (Model: ALS 1296, BioRad, USA and G-STORM, GSI, England, Serial no: GT-11620) was used along with the following PCR profile: an initial denaturation step for 5 min at 94°C (hot start and strand separation), followed by 34 cycles of denaturation (94°C), annealing (55°C) and primer elongation (72°C) for 30 seconds each and then a final extension at 72°C for 5 min. Amplified products were stored at -20°C until further use.

2.3. Electrophoretic Separation and Visualization of Amplified Products

Prior to electrophoresis, each PCR product was mixed with gel loading dye (bromophenol blue, xylene cyanol and sucrose) and electrophoresis was carried out in a mini vertical electrophoresis tank

(CBS Scientific Co Inc., CA. USA), run on 8% polyacrylamide gels in TBE buffer. Four microliters of the sample was loaded in each well and run at 80 Volts for 90 minutes. The gel, after electrophoresis, was stained with ethidium bromide for 30-35 min, kept in the dark, and then scanned using an UVPRO (Uvipro Platinum, EU) gel documentation unit linked to a PC. The reproducibility of amplification products was confirmed twice for each primer.

2.4. SSR Data Analysis

The size of most intensely amplified fragments was determined by comparing the migration distance of amplified fragments relative to the molecular weight of known size markers, 50 base pairs (bp) DNA ladder using Alpha-Ease FC 5.0 software (Alpha Innotech, USA). The number of alleles per locus, major allele frequency, gene diversity and PIC values were calculated using Darwin 3.2. The genotypes were scored for the presence and absence of the SSR bands throughout all 6 genotypes and the data were exported to binary data for the presence (1) or absence (0) or as a missing observation for further analysis with Darwin 3.2. Darwin 3.2 was used to construct an unweighted pair group method with arithmetic averages (UPGMA) dendrogram showing the distance-based interrelationship among the genotypes and principal component analysis (PCA) at about 1000 boothstrap.

3. RESULT

3.1. Overall Allelic Diversity

The Twenty primers were used across the six elite Africa rice genotypes for their characterization and discrimination, A total of 129 SSR primers (Table 1) were used to genotype the six varieties and to investigate the level of polymorphism among them. The 129 SSR marker sets were well distributed through the 12 chromosomes of rice. Of all the primers used, 108 markers were Polymorphic while 18 were Monomorphic for the six varieties. The number of polymorphic bands per locus ranges from 1 (RM165 and RM538 on chromosomes 1and 5 respectively) to 15(RM1376 on chromosome 8). Markers (RM147, RM284, RM519, RM555, RM3262, and RM5875) show both monomorphic and polymorphic bands. The six varieties produced a total of 492 alleles (Table 1) and the average number of alleles per locus was 3.81, ranging from 1 (RM165, RM168, RM175, RM285, RM349, RM409, RM348, RM455, RM538, RM559, RM1036, RM 3381, RM3790, RM6054, RM6990, RM7585 and RM19620) to 15 (RM1376 on chromosome 8) and most markers, 81 (62.79%), revealed 3 alleles. In the work of Botstein *et al.* (1980) informative levels of markers were defined: 35 (29.41%) level is highly informative, 76 (63.87%) reasonably informative and only eight (6.72%) slightly informative. The size of the detected alleles produced using the SSR primer sets ranged from 50 bp in RM3265 to 400bp in RM5095. This reflects a large difference in the number of repeats between the different alleles.

Table1. *The list of 129 SSR primers used in the estimation of genetic diversity of 6 rice genotypes, showing variation of alleles number (AN), Size of bands, monomorphic (MM) and polymorphic (PM) bands and PIC values.*

PRIMER NAME	CN	PRIMER SEQUENCE	AN	SIZE OF BANDS (BP)	MM	PM	PIC	GENE DIVERSITY
RM5	1	TGCAACTTCTAGCTGCTCGA (F) GCATCCGATCTTGATGGG (R)	3	110-120	-	3	0.2747	0.3333
RM6	2	GTCCCCTCCACCCAATTC (F) TCGTCTACTGTTGGCTGCAC (R)	4	170-190	-	4	0.2392	0.2778
RM7	3	TTCGCCATGAAGTCTCTCG (F) CCTCCCATCATTTCGTTGTT (R)	3	170-200	-	3	0.2402	0.3148
RM11	7	TCTCCTCTTCCCCCGATC (F) ATAGCGGGCGAGGCTTAG (R)	5	120-140	-	5	0.3200	0.4074
RM101	12	GTGAATGGTCAAGTGACTTAGGTGG C (F) ACACAACATGTTCCCTCCCATGC (R)	1	270	1	-	0.0000	0.0000
RM147	10	TACGGCTTCGGCGGCTGATTCC (F) CCCCCGAATCCCATCGAAACCC (R)	7	80-160	4	3	0.2549	0.3175
RM163	5	ATCCATGTGCGCCTTTATGAGGA (F) CGCTACCTCCTTCACTTACTAGT (R)	6	150-170	-	6	0.2998	0.3750

RM165	1	CCGAACGCCTAGAAGCGCGTCC (F) CGGCGAGGTTTGCTAATGGCGG (R)	1	180	-	1	0.2392	0.2778
RM 168	3	TGCTGCTTGCCTGCTTCCTTT (F) GAAACGAATCAATCCACGGC (R)	1	100	1	-	0.0000	0.0000
RM 175	3	CTTCGGCGCCGTCATCAAGGTG (F) CGTTGAGCAGCGCGACGTTGAC (R)	1	80	1	-	0.0000	0.0000
RM190	6	CTTTGTCTATCTCAAGACAC (F) TTGCAGATGTTCTTCCTGATG (R)	4	100-140	-	4	0.3191	0.4028
RM197	6	GATCCGTTTTTGCTGTGCCC (F) CCTCCTCTCCGCCGATCCTG (R)	4	190-220	-	4	0.3677	0.4861
RM202	11	CAGATTGGAGATGAAGTCCTCC (F) CCAGCAAGCATGTCAATGTA (R)	6	170-230	-	6	0.2890	0.3571
RM 205	9	CTGGTTCTGTATGGGAGCAG (F) CTGGCCCTTCACGTTTCAGTG (R)	5	120-130	-	5	0.3457	0.3445
RM206	11	CCCATGCGTTTAACTATTCT (F) CGTTCCATCGATCCGTATGG (R)	10	150-230	-	10	0.3060	0.3833
RM208	2	TCTGCAAGCCTTGTCTGATG (F) TAAGTCGATCATTGTGTGGACC (R)	5	170-190	-	5	0.2658	0.3194
RM212	1	CCACTTTCAGCTACTACCAG (F) CACCCATTTGTCTCTCATTATG (R)	3	110-140	-	3	0.3200	0.4074
RM 214	7	CTGATGATAGAAACCTCTTCTC (F) AAGAACAGCTGACTTCACAA (R)	2	110	-	2	0.2392	0.2778
RM 215	9	CAAAATGGAGCAGCAAGAGC (F) TGAGCACCTCCTTCTCTGTAG (R)	4	140-150	-	4	0.27315	0.33335
RM216	10	GCATGGCCGATGGTAAAG (F) TGTATAAAACCACACGGCCA (R)	6	130-180	-	6	0.3060	0.3833
RM228	10	CTGGCCATTAGTCCTTGG (F) GCTTGCGGCTCTGCTTAC (R)	7	110-200	-	7	0.3457	0.3492
RM 234	7	ACAGTATCCAAGGCCCTGG (F) CACGTGAGACAAAGACGGAG (R)	3	150	-	3	0.3102	0.3889
RM247	12	TAGTGCCGATCGATGTAACG (F) CATATGGTTTTGACAAAGCG (R)	4	150-180	-	4	0.3022	0.3796
RM248	7	TCCTTGTGAAATCTGGTCCC (F) GTAGCCTAGCATGGTGCATG (R)	4	75-100	-	4	0.3090	0.4444
RM 249	5	GGCGTAAAGGTTTTGCATGT (F) ATGATGCCATGAAGGTCAGC (R)	4	130	-	4	0.2925	0.3611
RM 261	4	CTACTTCTCCCCTTGTGTCG (F) TGTACCATCGCCAAATCTCC (R)	3	130-140	-	3	0.3102	0.3102
RM264	8	GTTGCGTCCTACTGCTACTTC (F) GATCCGTGTCGATGATTAGC (R)	7	170-180	-	7	0.3042	0.3810
RM276	6	CTCAACGTTGACACCTCGTG (F) TCCTCCATCGAGCAGTATCA (R)	3	90-150	-	3	0.3200	0.4444
RM279	2	GCGGGAGAGGGATCTCCT (F) GGCTAGGAGTTAACCTCGCG (R)	2	180-190	-	2	0.2392	0.2778
RM 284	8	ATCTCTGATACTCCATCCATCC (F) CCTGTACGTTGATCCGAAGC (R)	2	140-160	1	1	0.1196	0.1389
RM 285	4	CTGTGGGCCCAATATGTCAC (F) GGCGGTGACATGGAGAAAG (R)	1	190	1	-	0.0000	0.0000
RM286	11	GGCTTCATCTTTGGCGAC (F) CCGGATTCACGAGATAAACTC (R)	4	90-130	-	4	0.3603	0.4722
RM295	7	CGAGACGAGCATCGGATAAG (F) GATCTGGTGGAGGGGAGG (R)	2	180-190	-	2	0.2845	0.3519
RM 296	9	CACATGGCACCAACCTCC (F) GCCAAGTCATTCACTACTCTGG (R)	2	120-130	-	2	0.3457	0.4444
RM310	8	CCAAAACATTTAAAATATCATG (F) GCTTGTTGGTCATTACCATTC (R)	4	60-110	-	4	0.3102	0.3889
RM311	10	TGGTAGTATAGGTACTAAACAT (F) TCCTATACACATACAAACATAC (R)	8	140-190	-	8	0.3131	0.3958
RM315	1	GAGGTACTTCCTCCGTTTCAC (F) AGTCAGCTCACTGTGCAGTG (R)	3	140	-	3	0.3604	0.4722
RM316	9	CTAGTTGGGCATACGATGGC (F)	3	180-190	-	3	0.3102	0.3889

		ACGCTTATATGTTACGTCAAC (R)						
RM 317	4	CATACTTACCAGTTCACCGCC (F) CTGGAGAGTGTCAGCTAGTTGA (R)	3	140-160	-	3	0.2845	0.3519
RM332	11	GCGAAGGCGAAGGTGAAG (F) CATGAGTGATCTCACTCACCC (R)	4	170-200	-	4	0.3555	0.4630
RM336	7	CTTACAGAGAAACGGCATCG (F) GCTGGTTTGTTTCAGGTTCG (F)	9	180-230	-	9	0.3244	0.4111
RM340	6	GGTAAATGGACAATCCTATGGC (F) GACAAATATAAGGGCAGTGTGC (R)	4	170-190	-	4	0.2658	0.3194
RM341	2	CAAGAAACCTCAATCCGAGC (F) CTCCTCCCGATCCCAATC (R)	8	100	-	8	0.3134	0.3951
RM349	4	TTGCCATTCGCGTGGAGGCG (F) GTCCATCATCCCTATGGTCG (R)	1	140	1	-	0.0000	0.0000
RM402	6	GAGCCATGGAAAGATGCATG (F) TCAGCTGGCCTATGACAATG (R)	4	150-170	-	4	0.3102	0.3889
RM408	8	CAACGAGCTAACTTCCGTCC (F) ACTGCTACTTGGGTAGCTGACC (R)	2	120	1	1	0.3457	0.4444
RM 409	9	CCGTCTCTTGCTAGGGATTC (F) GGGGTGTTTTGCTTTCTCTG (R)	1	80	1	-	0.0000	0.0000
RM 410	9	GCTCAACGTTTCGTTCCTG (F) GAAGATGCGTAAAGTGAACGG (R)	12	180-300	-	12	0.2925	0.414
RM447	8	CCCTTGTGCTGTCTCCTCTC (F) ACGGGCTTCTTCTCCTTCTC (R)	3	110-130	-	3	0.3031	0.3778
RM 348	4	CCGCTACTAATAGCAGAGAG (F) GGAGCTTTGTTCTTGCGAAC (R)	1	140	1	-	0.0000	0.0000
RM455	7	AACAACCCACCACCTGTCTC (F) AGAAGGAAAAGGGCTCGATC (R)	1	140	1	-	0.0000	0.0000
RM510	6	AACCGGATTAGTTTCTCGCC (F) TGAGGACGACGAGCAGATTC (R)	4	110-130	-	4	0.3191	0.4028
RM519	12	AGAGAGCCCCTAAATTTCCG (F) AGGTACGCTCACCTGTGGAC (R)	4	150-170	3	1	0.2925	0.3610
RM538	5	GGTCGTTGAAGCTTACCAGC (F) ACAAGCTCTCAAAACTCGCC (R)	1	100-290	-	1	0.2392	0.2778
RM 542	7	TGAATCAAGCCCCTCACTAC (F) CTGCAACGAGTAAGGCAGAG (R)	2	80	-	2	0.2924	0.3611
RM555	2	TTGGATCAGCCAAAGGAGAC (F) CAGCATTGTGGCATGGATAC (R)	3	220-250	1	2	0.1794	0.2083
RM 559	4	ACGTACACTTGGCCCTATGC (F) ATGGGTGTCAGTTTGCTTCC (R)	1	160	1	-	0.0000	0.0000
RM584	4	AGAAAGTGGATCAGGAAGGC (F) GATCCTGCAGGTAACCACAC (R)	5	180-200	-	5	0.2658	0.3194
RM 590	10	CATCTCCGCTCTCCATGC (F) GGAGTTGGGGTCTTGTTCG (R)	3	150-170	3	-	0.0000	0.0000
RM 1026	9	GCCTCTGGCAGAATAGCATC (F) TATCACTTTGCTGCCTAGGC (R)	9	150-170	-	9	0.3244	0.4111
RM1036	12	CTCATTTGTCGATTGCCGTC (F) ATGGGAGGAGTGATCAAACG (R)	1	80	1	-	0.0000	0.0000
RM1126	10	AGAAAAGGCTGCATCAGTGC (F) TCCAACGACAGACTGTACGG (R)	4	130-180	-	4	0.3750	0.5000
RM 1256	3	ACGCGAAGCAACGGAGATAG (F) CTAGCCTCGATGCGAAAAAC (R)	4	130-150	-	4	0.3457	0.4444
RM1364	7	AAGAAATTCAAAACACATGA (F) AAAACATCTACTTTGATCCA (R)	1	130	1	1	0.0000	0.0000
RM1370	6	AAACGAGAACCAACCGACAC (F) GGAGGGAGGAATGGGTACAC (R)	4	130-160	-	4	0.2890	0.3571
RM1375	10	CTACACGCGCAAACTCTGTC (F) ATGAAGGTCTAGGCTGCACC (R)	7	160-190	-	7	0.3253	0.4136
RM1376	8	CATGTGTGATGACTGACAGG (F) GGTGCTGTGATGATTCTTTC (R)	15	100-150	-	15	0.3060	0.3833
RM1880	12	ACCACTAAATAAGCACATAC (F)	6	90-110	-	6	0.2570	0.3056

		GGCATCATACATTAAAATAC (R)						
RM 2186	11	CTAATATTAGCCATGAAACA (F) CTTATCAGTAGAACTGCAGA (R)	2	60-90	-	2	0.2392	0.2778
RM2504	10	TAACACAACAATAGCGTCAG (F) TAGGAAGAACTGAAGAAGCA (R)	14	160-250	-	14	0.2883	0.3547
RM2851	12	CTAATATTAGCCATGAAACA (F) CTTATCAGTAGAACTGCAGA (R)	3	90	-	3	0.3200	0.4075
RM3202	3	TTCACTTCCTATTGGCGGC (F) TCATCATCAGTCCAGCATCG (R)	5	190-220	-	5	0.3603	0.4722
RM3252	1	GGTAACTTTGTTCCCATGCC (F) GGTCAATCATGCATGCAAGC (R)	3	170-210	-	3	0.3264	0.4167
RM 3262	8	ACCGATGAGCTCTCCACATC (F) TGACCTCACTTCACTTCCCC (R)	2	180-200	1	1	0.1875	0.2500
RM3265	3	TCTGTTGTTGTTCTGCCTGC (F) CCAGTAAAGCATCAGCCCTC (R)	3	60-70	-	3	0.375	0.5000
RM3343	6	GTTTCGCGAAGCCCTCTC (F) AAACCCTAACCCTCGACTCC (F)	2	150-160	-	2	0.3603 5	0.4722
RM3381	5	ACAAGCACCAGCCACAATAG (F) GGTGTTGTTTTGGACGAACG (R)	1	120	1	-	0.0000	0.0000
RM 3392	3	GTCCAATGATTCGTTCCCAC (F) CTTCACCGTTCACCAATTCC (R)	6	150-200	-	6	0.2348	0.2870
RM3414	6	TAGGGCAATTGTGCAAGTGG (F) TTGGGAATTGGGTAGGACAG (R)	3	70-100	-	3	0.3102	0.3889
RM3448	12	CTTCCTCCTTCCTCCTCCTC (F) CACGTGACACGTACACCCTC (R)	12	130-190	-	12	0.2481	0.2658
RM3486	5	TCTCTTTTCCCTCCTTTCCC (F) GGCCTGCAAGAGGAGAAAAC (R)	2	100	-	2	0.2747	0.3333
RM3702	8	TCTGAAATAGAAGCTCAGCA (F) GAAAGTTATTGCACTCTCCA (R)	5	140-160	-	5	0.3603	0.4020
RM3743	7	TAGCCTTGTTCCATCCATCC (F) CTTCTCCCTCTCCTCCTTCC (R)	3	170	-	3	0.3555	0.3743
RM3746	1	AAATGGGCTTCCTCCTCTTC(F) CAGCCTTGATCGGAAGTAGC (R)	4	75-110	-	4	0.2731	0.3333
RM3790	5	TAATTGCGGTCTCGTGCC (F) AACCACCTCAACTACTGCCG (R)	1	110	1	-	0.0000	0.0000
RM5095	10	CTATATGACTATGCGAATGG (F) ACAAATGCAACTAAGGTAGA (R)	10	170-400	-	10	0.2847	0.3500
RM 5428	8	ATGCAATACAGCACACTCGC (F) CTTATGCTCTCATGGCTCCC (R)	6	200-240	-	6	0.3244	0.4074
RM5526	9	TCAGCCTGGCCTCTCTTATC (F) ATGATCCTCCACCCACTAGC (R)	4	170-190	-	4	0.3191	0.4028
RM5543	7	ACCACTTGCTGGAATCCTTG (F) GCAAATTCTGGGCTATCTGC (R)	4	190-230	1	3	0.2400	0.3056
RM5590	11	TGGATAAGCGATTGAGGTAG (F) CGTTATAATGAGGGAGGGAG (R)	2	150-170	-	2	0.3337	0.4306
RM5599	11	CTCACAATATCACCATCCAC (F) AATTTTGTGCTGTTGTTGAA (R)	4	100-140	-	4	0.3750	0.5000
RM5746	12	TCGCTACGTCGACTGATTTG (F) ATATCATCAGTCGGCAGCAG (R)	3	150-190	-	3	0.2796	0.3343
RM5799	9	ATCGAACCATCCAGGATGAC (F) TTGCACAAGAGGCAACACTC (R)	3	170-180	-	3	0.2605	0.3111
RM5812	2	CGCTGACATCTTGCCCTC (F) GTAGGACCCACGTGTCATCC (R)	8	130-140	-	8	0.3264	0.4167
RM 5875	7	TTTCCCACCAGAGGAAGATG (F) AAGTTCCCAAGTTGGATCCG (R)	2	80-90	1	1	0.1729	0.2222
RM5926	11	ATATACTGTAGGTCCATCCA (F) AGATAGTATAGCGTAGCAGC (R)	7	160-190	-	7	0.3302	0.4222
RM 5979	4	TGCTGGACCTCACTGTTCTG (F) ACGTGGCTCAATCAGGAAAC (R)	2	150-160	-	2	0.3604	0.4021
RM	5	CCCTCCGTACGGATACACAC (F)	1	110	1	-	0.0000	0.0000

6054		CTCTTCGGCTTCATCTCCTC (R)						
RM6296	12	TCTTGCCTCGCTAGGGTTAG (F) CCCACGTTTCTCTTGTCCTC (R)	3	150	3	-	0.0000	0.0000
RM6314	4	GATTCGTGTCGGTTGTCAAG (F) GGTTCAGGGACGAATTTCAG (R)	2	150-175	-	2	0.2747	0.3333
RM6335	11	CAAGTTTACGGCAGCTAGGC (F) GAGTGGAGCACAAGGAAAGG (R)	2	160-180	-	2	0.3200	0.4074
RM6775	6	GCAGATCAAGTATGCCTGCC (F) TCGCTAGATAGGGGATGTGG (R)	2	180-200	-	2	0.3200	0.4074
RM6797	9	CCTCCTCCATCAGGATCATC (F) GCTAGGTTGAATGCCCGTAC (R)	2	200	2	-	0.0000	0.0000
RM 6990	8	GGTGTGATCCTTTCTGATGC (F) ACGGGTGTGATCCCAGATAC (R)	1	130	1	-	0.0000	0.0000
RM7000	3	CCCTTCTTTTCAACTGAATA (F) TTGTAACAATGAACTCGTTC (R)	7	130-250	-	7	0.3084	0.3889
RM7075	1	TATGGACTGGAGCAAACCTC (F) GGCACAGCACCAATGTCTC (R)	2	120-140	-	2	0.2392	0.2778
RM7173	11	GAGCGTTTTTAGGATGCCAC (F) GTGATGTCGGATTCTTGGTG (R)	2	120-130	-	2	0.3071	0.3888
RM7187	4	CAGCGAACGTGGTGTCTTC (F) CCCACACCAACTTCTCGC (R)	2	160-180	-	2	0.3457	0.4444
RM7252	2	GGAGGAGGAGAAGGGTTTTG (F) ACGCGCTGTCAAGTTAAAGG (R)	2	170	-	2	0.2392	0.2778
RM7293	5	CCTAGGGGATCCAAGATGTC (F) GCACGGATCTACATACATGC (R)	3	150	-	3	0.2998	0.3750
RM 7356	8	CCAAGGACACATATGCATGC (F) GCAATTCATGGCGCTGTTC (R)	2	170-200	-	2	0.3457	0.4444
RM7382	2	GCTCCTCGAATCTGTCGATC (F) CACTCCGAACTCCTACGCTC (R)	2	170-180	-	2	0.2747	0.3333
RM 7485	2	GCCAGTTTCTCCAAAAGACG (F) AACTAGCCTCGACAGCGAAC (R)	5	160-180	-	5	0.2731	0.3333
RM7492	10	AGATGGTTGCCAAGAGCATG (F) GTCACGTGGCGATTTAGGAG (R)	3	130-310	-	3	0.3022	0.3796
RM7576	3	CTGCCCTGCCTTTTGTACAC (F) GCGAGCATTCTTTCTTCCAC (R)	6	200-300	-	6	0.3361	0.4333
RM7585	4	CCTCCTCCCTCGACTACCTC (F) GGTGTGTCGGTGTGATATGC (R)	1	150	1	-	0.0000	0.0000
RM7642	3	ACGAAATATCAGGGCACCTG (F) GTTGACTTTGGTCATGAGGG (R)	3	100-150	-	3	0.3530	0.4583
RM8004	1	TTGACCAAAGGTGATTGTAAT (F) CTTGATGAGTTTCATGAGCA (R)	2	110-150	-	2	0.2818	0.3444
RM8243	8	CTCGTGCAACCATTATATTC (F) ACCTTAGCTGTCCTGAATTG (R)	2	190-210	-	2	0.3191	0.4028
RM11893	1	AATTCGGTCACTCGCTGTCACG (F) CTGCGGACGAAATTGCTTAGCC (R)	2	150-160	-	2	0.3530	0.4583
RM14001	2	TGTGGCTGGGCTCCGATACC (F) ACCCTGCAGGATCATCAGAACG (R)	2	130	-	2	0.2392	0.2778
RM18349	5	CGTAAACACGAGCACACAAAGG (F) ACACACAACAGCTGCTCACTGG (R)	3	80-90	-	3	0.31997	0.4074
RM 18614	5	TGCTACCGATAGTAGAAGTGATCG (F) GCATGTGTACAGGAGGAAGC (R)	8	150-190	-	8	0.26583	0.3195
RM 19218	5	CGGAGGGAGTAGGTACGTAGGG (F) CCCATTCCATTCTACACTGACG (R)	4	160-200	-	4	0.2392	0.2778
RM19620	6	GCGACGAGGAAGAAGATTAGTTCG (F) GCGGCACTTCGAGCAGTACG (R)	1	170	1	-	0.0000	0.0000
RM 26009	6	CGAGCAGCTGTGTGGAGTTGTGC (F) ACGACGAAGGTGGCAAGTCACG (R)	2	170-180	-	2	0.3200	0.4074
RM2606	11	GATCCATATGCCTCTTCGATTGG (F)	4	120-150	-	4	0.2924	0.3418

3		AACTCCAGCAGTGAGAGCGTAGC (R)					5	
RM26998	11	ACGCACGCACATCCTCTTCC (F) CGGTTCTCCATCTGAAATCCCTAGC (R)	7	120-230	-	7	0.3347	0.4286
RM 28130	12	CAGCAGACGTTCCGGTTCTACTCG (F) AGGACGGTGGTGGTGATCTGG (R)	3	170-190	3	-	0.0000	0.0000

Table1 CN: Chromosome number; AN: Alleles number; MM: Monomorphic bands; PM: Polymorphic bands; PIC: polymorphic information content.

The average gene diversity over all SSR loci was 0.310768 and the PIC value for the SSR loci was 0.250136. The lowest gene diversity 0.0000 was recorded in RM101, RM168, RM175, RM285, RM349, RM409, RM348, RM455, RM559, RM1036, RM1364, RM3381, RM3790, RM6054, RM6296, RM6797, RM6990, RM7585, RM19320 and RM28130 while the highest gene diversity 0.5000 was recorded in primers RM1126, RM3265 and RM5599. The PIC value for each marker was used to assess the polymorphic level. Primers RM101, RM168, RM175, RM285, RM349, RM409, RM348, RM455, RM559, RM1036, RM1364, RM3381, RM3790, RM6054, RM6296, RM6797, RM6990, RM7585, RM19320 and RM28130 gave the lowest PIC value of 0.0000 while primers RM1126, RM3265 and RM5599 gave the highest PIC value of 0.3750. Primer RM1376 gave the highest number of polymorphic bands and has the highest number of alleles. Overall, the 129 markers provided sufficient polymorphism information for evaluation of genetic diversity of the six rice varieties.

3.2. Phylogenetic Reconstruction of the Six Varieties of Rice Based on the Allelic Variation at SSR Loci

DNA Amplified by PCR was subjected to electrophoresis and Separation of alleles on polyacrylamide gel followed by staining with ethidium bromide and visualized under UV light (Figure 1) shows the result obtained from using two SSR markers. The genetic relationship among the six rice varieties is presented in a dendrogram based on informative microsatellite alleles (Figure 2) with minimum dissimilarity value of 0.3644 and maximum dissimilarity value of 0.6923. The six rice varieties were separated into 3 groups at 60% dissimilarity level. The SSR-based dendrogram separated NERICAL-L-34 variety from all the other rice varieties in the first cluster. However, the second cluster separated into two sub-clusters: the first sub-cluster contained BW-348-1 and FARO-44 while the second sub-cluster contained two groups, WITA-4 being in the first group and the second group is separated into two subgroups consisting of NERICA-L-19 and FARO-57. The principal component analysis (Figure 3) done at 1000 bootstraps further confirms the grouping of the six varieties as indicated in the dendrogram. Plate 1 gave a visual representation of the variation in the height of the six rice genotypes under study.

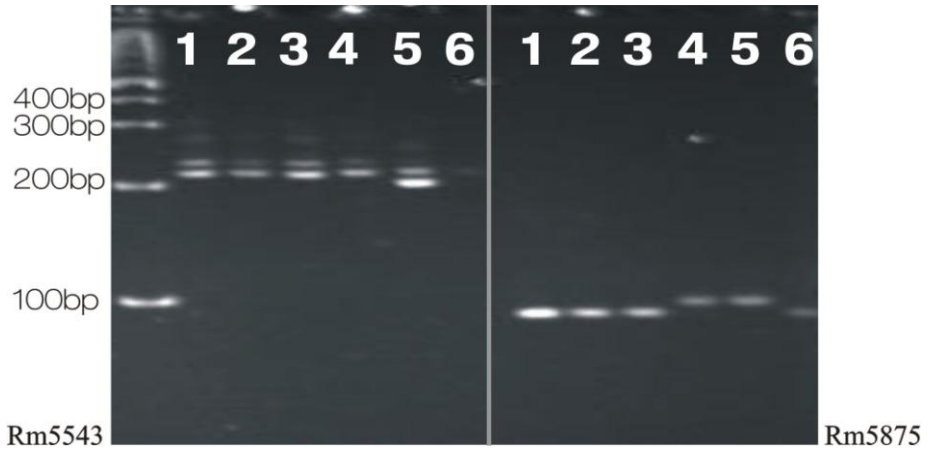

Figure1. *Separation of alleles on polyacrylamide gel followed by staining with ethidium bromide and visualized under UV light. PCR products were amplified with rice SSR primers RM 5543 (left block) and RM5875 (right block). The lane marked with band sizes is the ladder marker. All were 100 base pair (bp). 1, 2, 3, 4, 5 and 6 represent elite Africa lowland rice varieties; WITA-4, BW-348-1, FARO 44, FARO 57, NERICA-L-19 and NERICA-L-34.*

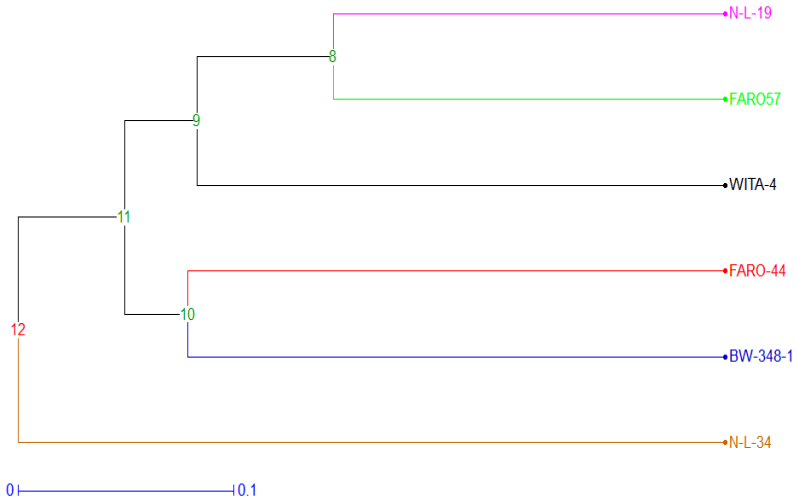

Figure2. *Phylogenetic reconstruction of 6 varieties of Rice*

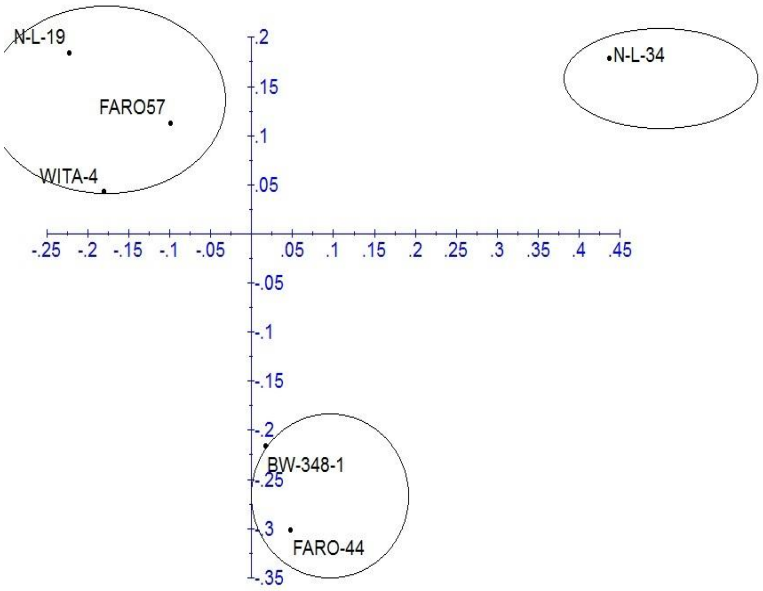

Figure3. *Principal Component Analysis (PCA) of the six rice genotypes*

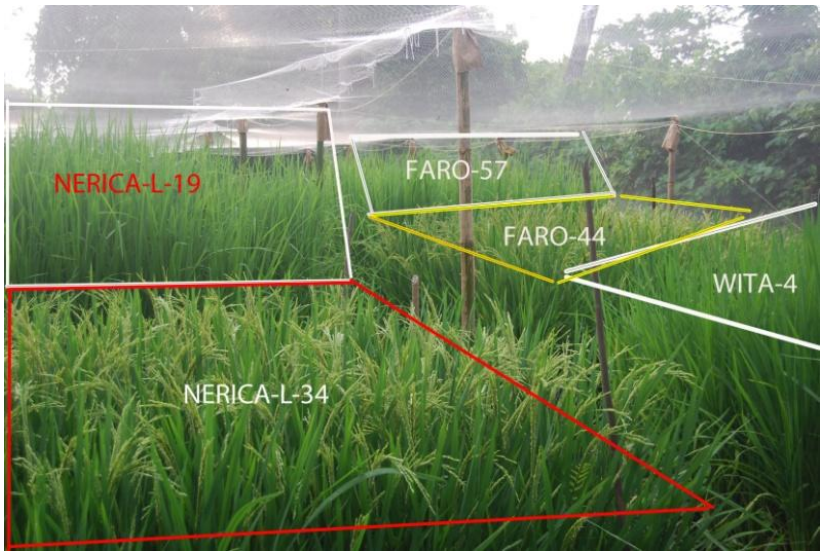

Plate1. *Visual variation in the height of the six rice genotypes*

4. DISCUSSION

A good isolation protocol should be simple, rapid and efficient, yielding appreciable levels of high quality DNA suitable for molecular analysis (Xiaohua *et al.,* 2010). CTAB-A method used for extraction of Genomic DNA of the six rice genotypes produced a high quality DNA which was free from contaminants including carbohydrates, phenol, aromatic compounds and RNA. High quality DNA produces clear Bands which were scored and used to determine gene diversity, size of the base pairs and PIC values of the various primers used. The DNA quality values for the six rice genotypes ranges between 1.94 and 2.03 using ratio values of A260/A280. This values were contrary to the result of Xiaohua *et al.,* (2010) when CTAB-A was used for DNA extraction of *Reaumuria soongorica* where DNA quality values obtained were between 1.94 and 2.3 ratio values of A260/A280. But a lesser value which ranges from 1.7- 2.02 was recorded when CTAB-B method of DNA extraction was used and this produced Genomic DNA of better purity. The reason for disparity in the result obtained from using CTAB-A isolation protocol for six lowland rice genotypes and *R. soongorica* may be due to the leaves of *R. soongorica* which have evolved into the form of pellets suitable for arid environment. The leaves were very hard in texture and contain high level of polysaccharides, polyphenols and secondary metabolites that co-precipitate with DNA, making DNA isolation difficult.

In the study of genetic diversity, physiological, morphological and molecular data are usually harvested, analysed and used. Molecular diversity is based on the naturally occurring polymorphism which escapes the limitations of environmental influences and gene expression. On the contrary, according to Bruschi *et al.* (2003), both the morphological and physiological traits are largely influenced by environmental conditions and cultural practices. Mamunur *et al.* (2011) in their investigation of genetic diversity of some rice varieties using morphological, physiological and molecular data also concluded that information provided by molecular data using SSR markers is more reliable. Hence, molecular data should be given preference over morphological and physiological data in the investigation of genetic diversity among organisms.

SSR markers have been commonly used in evaluating genetic diversity and phylogenetic relationship among organisms because of their abundance in genomes and high allelic polymorphism, co-dominance, and easy manipulation by PCR. Nantawan *et al.* (2011), in their comparison between SSR and RAPD-based information on genetic diversity, revealed that SSR markers detected higher polymorphism (89.47%) when compared with RAPD Markers (68.94%). This is consistent with the study of Mahmoud *et al.* (2005) where the effectiveness of SSR, RAPD and AFLP markers in determining the marker system with the highest level of polymorphism amongst seven Egyptian rice varieties was investigated. SSR gave the highest level of polymorphism (90%) followed by RAPD (72.9%) and AFLP (67.9%). However, Mahmoud *et al.* (2005) further stated that SSR data were less informative in characterizing closely related Egyptian rice genotypes when compared with RAPD and AFLP.

In this study, the SSR markers showed high levels of polymorphism among the six rice varieties. A total of 108 polymorphic and 18 monomorphic alleles (83.72% polymorphism) with an average number of alleles of 3.81 per locus (range: 1-15 per locus) were recorded (Table 1). These values were comparable to those reported earlier. Similar values (3.74 alleles per locus; 2-14) were recorded by Jiangbo *et al.* (2011). Here, 152 polymorphic SSR markers were used to genotype 128 japonica varieties. Mahmoud *et al.* (2005) recorded 5 alleles per locus; range 2-8 with the use of six primers to estimate genetic relationship among seven Egyptian rice varieties. Also, Bounphanousay *et al.* (2008) recorded 4.3 alleles per locus; range 2-9 and Zeng *et al.* (2004) recorded a lower value of 3.1 alleles per locus; range 2-7. On the contrary, other reports showed higher values. In an investigation carried out by Giarrocco *et al.* (2007) using 26 SSR loci to estimate genetic relationship among 69 Argentine rice accessions, a higher value of 8.42 alleles per locus; range 3-21 was recorded. Jayamani *et al.* (2007) also reported a similar value of 7.7 alleles per locus; range 3-16 from a fingerprinting study of 178 Portuguese rice accessions at 24 SSR loci. Other reports, however, showed either lower or higher allelic diversity. Brondani *et al.* (2002) reported much higher value (14.6 alleles per locus; range 6-22) from 192 accessions of Brazilian landrace rice.

The reason for the wide variation in the number of alleles detected was due to the difference in the sets of germplasms, number of genotypes, number and distribution of SSR loci and methods of gel electrophoretic detection in different studies. The low number of alleles was usually obtained from a

collection of breeding lines and closely related cultivars such as those used in Zeng *et al.* (2004). High number of alleles was expected to be found when a large number of landraces from a wide range of geographical origins are included in the study (Brondani *et al.*, 2002).

5. CONCLUSION

The molecular characterisation of the six rice varieties carried out using simple sequence repeat (SSR) marker system with 129 primers that cut across the 12 chromosomes of rice, shows clear differences among the plant materials, each occupying a taxon of its own. The few similarities observed in the genetic make-up as revealed by the molecular characteristics of the rice varieties using SSR markers are only an indication that they share a common ancestry.

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