Original Research Article

Genomic DNA diversity analysis of some accessions of *Oryza sativa* L. for breeding and conservation strategies.

Abstract: Molecular characterization provides basic vital information on the degree of genetic diversity among genetic materials which guides development of breeding programme and inform strategies on genetic conservation of important accessions. A total of 21 SSR markers were used to characterize 25 rice genotypes. The 21 microsatellite markers were highly polymorphic and allele number per locus ranged from 3 alleles (RM 224, RM 229, RM 256, RM 413, RM 3, RM 276, RM 219, RM 88 and RM 349) to 5 alleles (RM 247 and RM 288), with a mean of 3.67 alleles through the 21 loci obtained in the study. The content value of the polymorphic information ranged from 0.372 in RM 88 to 0.503 in RM 256 with overall average of 0.422 in all the 21 loci. PIC values indicated that RM 256 was the best marker for the identification of the rice genotypes. The main allele frequency at each locus ranged from 54.5% (RM 219) to 69.3% (RM 229). The pairwise genetic dissimilarity co-efficient showed that the highest genetic distance was obtained between NERICA 2 and MR 220; NERICA 2 and MR 263; and finally between WITA 4 and NERICA 2 (76.0%). The lowest genetic dissimilarity was between NERICA 6 and NERICA 8 (19.0%) followed by WITA 4 and NERICA L 34; BW 348-1 and IWA 10 (24.0%). Intercrossing far related genotypes could yield hybrids of high heterosis using SSR marker**.** Cluster analysis revealed reasonable levels of locational groupings among the genotypes.

Keywords: Conservation, Diversity, Genomic DNA, Heterosis, Rice

Introduction

Most often morphological traits are controlled by polygenes and quantitative agronomic traits are used to measure differences among populations in order to resolve complex genetic issues like stress tolerance and yield potential. Morphological traits are relatively less expensive but not as reliable as DNA fingerprinting techniques for precise discrimination and analysis of closely related species. Notwithstanding, morphological traits still remains useful for preliminary evaluation of genetic materials because it is relatively simple, less expensive and applicable as a general approach for assessing genetic diversity among morphologically different accessions (Beyene et al., 2005). Therefore, morphological characterization becomes the most convenient and sustainable method of taxa discrimination in developing countries than other methods. Farmers can easily understand and adopt results from studies based on morphological data compared to other methods. According to Nassir and Ariyo (2007), the direct identification of character expression with particular genotypes under real-time field conditions remains a unique point for field-based multivariate analysis compared with laboratory techniques which classify variability at the level of DNA. On the contrary, molecular techniques are useful for quantification of genetic variation and characterization of genetic resources. An assessment of genetic diversity based on morphological and agronomic traits might be misleading, due to the influence of environment and apparent distinct genotypes may be a consequence of mutation

with a common genetic base. Detection of DNA variation and characterization of accessions by molecular methods is a major advantage in the direct investigation of genotypic information by excluding environmental influences. In addition, molecular methods can be used at early plant growth stage which saves costs and time. Molecular markers can therefore complement existing estimation of phenotypic diversity for the construction of core collection in the management of Gene bank (Hammer, 2004). In rice breeding programmes, molecular markers could serve as an assessment tool for the effective determination of genetic relatedness among selected lines. Also, the tool is useful in the quantification of allelic diversity in crop with potential to provide distinctive DNA fingerprints for every genetically dissimilar genotype which is important in the identification of differences in cultivars. At protein and DNA levels the assessment of genetic diversity among germplasm has been achieved through protein and molecular marker techniques (Mueller and Wolfenbarger, 1999). Several DNA amplification methods which include: Protein based markers i.e. isozymes (Market and Moller, 1959) DNA based markers e.g. Restriction Fragment Length Polymorphism (RFLP) (Botstein *et al.,* 1980), Microsatellite or Simple Sequence Repeat (SSR) (Tautz and Renz, 1984) Random Amplified Polymorphic DNA (RAPD) (Williams et al., 1990) and Amplified Fragment Length Polymorphism (AFLP) (Zabeau and Voss, 1993) have been reported in literature. Simple sequence repeat is an important tool for genetic variation identification of germplasm (Powell et al., 1996; Ma et al., 2011; Sajib et al., 2012). It has some advantages such a simplicity, quickness, rich polymorphism and stability which is generally applied in genetic diversity analysis, molecular map construction and gene mapping (Ziang et al., 2007; Ma et al., 2011; Sajib et al., 2012), also in gene purity test (Peng et al., 2003; Ma et al., 2011; Sajib et al., 2012), fingerprint construction and analysis of germplasm diversity (Zhou et al., 2003; Xiao et al., 2006; Jin et al., 2010; Ma et al. 2011 and Sajib et al., 2012). There are two basic conservation strategies, each composed of various techniques that the conservationist can adopt to conserve genetic diversity once it has been located. The two strategies are *Ex situ* and *In situ*. *Ex situ* conservation means the conservation of components of biological diversity outside their natural habitat. It involves techniques such as Seed Storage, Field Gene Banks, *In Vitro* Storage, DNA Storage, Pollen Storage and Botanical Gardens. While *In situ* conservation means the conservation of ecosystem, natural habitat and maintenance and recovery of valuable populations of species in their natural surroundings where they have developed their distinctive properties. On-farm conservation and genetic reserves are forms of *In situ* conservation (Ajiboye 2013). The existing rice biodiversity in Nigeria can be properly conserved by giving unwavering attention to careful management and proper documentation to bridge the gap between germplasm available and germplasm utilized in breeding programs. There should be rice germplasm collection with a sense of mission to unexplored and under explored regions of the country while effort to conserve genetic resources such as released varieties, breeding lines, land races etc. at research institutes is accelerated. Rice germplasm should be assigned national accession numbers after eliminating the duplicates through systematic evaluation. Also, attribute based core collection of indigenous rice should be developed. A practical mechanism for *in situ* conservation through farmer participatory breeding approach and through proper incentives to farmers should be put in place. The current study focuses on the characterization of twenty-five rice genotypes with a view to determining the genetic relationship and diversity among the genotypes which could provide more information on conservation strategies and rice breeding programmes.

Materials and Methods

Germplasm collection and extraction of genomic DNA from rice leaves

Twenty-five genotypes of rice (Table 1) collected from three countries in Africa and Asia were raised for 12 days in the nursery of the Teaching and Research farm of Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria. Total genomic DNA was isolated from the leaves of twelve days' old seedlings using the CTAB (Cetyl trimethyl ammonium bromide) method as reported by Dellapota et al., (1983). Twenty-one Simple Sequence Repeat (SSR) markers representing the whole rice genome associated with the quality of grain and yield component traits were selected from the available framework map (Akagi et al., 1996; Temnykh et al., 2000). The extracted DNA quantification was done by NanoDrop^{TM} 1000 Spectrophotometer and isolated DNA was checked for homogeneity, intactness and purity using electrophoresis in 0.8% agarose gel.

Polymerase Chain Reaction (PCR) Amplification

PCR reactions were done in programmable Thermo Cycler (PTC) MJ research Inc. USA. The reaction volume was 15 µl which contained 2.0 µl of genomic DNA, 1x assay buffer, 200 µM of dNTPs, 2 µM $Mgcl_2$, 0.2 µM each of primer and 1 unit of *Taq polymerase*. Cycles' temperature was programmed as 95°C for 2 minutes followed by 94°C for 45s and 55°C for 1 minute then 72°C for 1 and half minutes for 35 cycles with an additional temperature of 72°C for 10 minutes' extension and 4°C to cool.

Amplified PCR was separated using 3% agarose gel prepared in Ethidium bromide stained 1x TBE buffer at a voltage of 90 V for a period of 45 minutes to an hour. Gel was visualized in UV trans-illuminator and photographs were taken using Alpha Digidoc gel documentation instrument. With each primer, clearly resolved and unambiguous bands were scored visually for the presence or absence of DNA. The scores were obtained in matrix form with '1' or '0', indicating the presence and absence of bands in each genotype.

SSR data analysis

Size of most strongly amplified fragments was determined by comparing the migration distance of amplified fragments relative to the molecular weight of known size markers, 100bps (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 PowerMaker version 3.25 (Liu and Muse, 2005) also, PIC analysis to be described and formula for its computation (Sharma et al., 2009) as shown below;

$$PIC = \sum x_i^2$$

Where x_i is the relative frequency of the ith allele of the SSR loci.

All the genotypes were scored for the presence and absence of the SSR bands throughout all the 25 rice 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 NTSYS-pc version 2.2 (Rohif, 2002). This was used to construct a UPGMA (Unweighted pair group method with arithmetic averages) dendrogram showing the distance-based interrelationship among the genotypes.

Results

DNA Quantification and Quality assessment

The result of DNA quantification and quality measurement from Nanodrop photometer is presented in Table 2. Twenty five rice genotypes were used for the molecular work, 21 SSR primers were selected from the published rice microsatellite framework map that showed polymorphism for the assessment of genetic diversity across selected rice genotypes. From the quantification results in Table 2, the ratio of values of 260/280 absorbance ranged from 1.73 – 2.01 demonstrating high purity of the DNA; an indication that the DNA used for this experiment was free of protein, polysaccharides and other contaminants. The Twenty two Traits linked microsatellite (SSR) markers used to assess genetic diversity among 25 rice genotypes with forward and reverse sequences of nucleotides were presented in (Table 3) with repeat motif of the SSR markers, location and number of repeats as previously published (http://www.gramene.org).

Allelic and gene diversity

Twenty one primers were used across 25 rice genotypes for their characterization and discrimination for both upland and lowland rice genotypes (Table 4). The highest number of alleles (5.0) was detected in the locus of primer RM 247 and RM 288 and the lowest number of alleles (3.0) was detected on each locus of RM 224, RM 229, RM 256, RM 413, RM 3, RM276, RM 219, RM 88 and RM 349. On the average 61% of the 25 rice genotypes shared common major allele at random locus ranging from 54.5% (RM 219) to 69.3% (RM 229). A moderate level of gene diversity exists across the studied rice genotypes and ranged from 0.38 to 0.55 with a mean value of 0.48. Figures 1-7 shows gel images of amplified fragments produced by primers RM256, RM 413, RM 3, RM 219, RM 304, RM 226 and RM 349 which suggested that these markers could be potentially used for molecular characterization of both upland and lowland rice from diverse sources.

Polymorphic information content (PIC) value

SSR markers were highly informative and polymorphic as evident from its PIC value which is a measure of polymorphism among genotypes for a marker locus used in linkage analysis. The PIC value of each marker which can be evaluated on the basis of its alleles, varied greatly for all tested SSR loci from 0.369 to 0.503 with mean value of 0.422 (Table 4). The highest PIC value 0.503 was obtained for RM 256 followed respectively by RM 304 (0.465), RM 3 (0.465), RM 413 (0.455), RM 219 (0.447), RM 349 (0.446) and RM 226 (0.445).

Pairwise genetic dissimilarity

A dissimilarity matrix was used for the determination of the level of relatedness among studied genotypes. The pairwise genetic dissimilarity indices (Table 5) indicated that the highest genetic dissimilarity was between FADAMA 42 and WITA 4 (76%), NERICA 2 and IR07A (76%), MR 263 and LAC 23 (76%), NERICA 2 and MR220 (76%), NERICA 2 and MR 263 (76%), as well as between WITA 4 and NERICA 2 (76%). These pairs were followed by FARO 57 and FADAMA 42 (71%), MR 220 and FADAMA 42 (71%), LAC 23 and IRRI 154 (71%), LAC 23 and IWA 8 (71%), MR 269 and LAC 23 (71%), NERICA 2 and MR 269 (71%), NERICA 6 and IRRI 154 (71%); NL 34, FADAMA 42, NERICA 2, and NERICA 6 (71%); OS6, IR07A, IRRI 154, IWA 8, MR 263, NL 30 and NL 34 (71%), UPIA and OS6 (71%), WITA 4 and LAC 23 (71%) and declining thereafter. The lowest genetic dissimilarity among the rice genotypes was

between NL 19 and FARO 57 (5%); this is followed by NL 19 and LADY'S FINGER (10%) and between FARO 57 and LADY'S FINGER (14%) respectively.

Cluster analysis

An unrooted neighbor-joining tree showing the genetic relationships among exotic and Nigerian elite genotypes obtained from SSR marker analysis is presented in Figure 8. Broadly, the cluster analysis distributed the twenty five genotypes into five major clusters. The first group (cluster I) consists of three genotypes (NERICA L 34, INPARI 10 and WITA 4), cluster II comprised of four individuals (MR 269, FARO 44, MR 263 and MR 220), cluster III recorded the highest number of 9 genotypes (IR07A, IR06N, IR06A, OS 6, NERICA 2, NERICA 8 Lac 23, NERICA 6 and FADAMA 42) representing 36% of the genotypes studied. Cluster IV consists of NERICA L 30, IWA 8, UPIA and IRRI 154 while cluster V consisted of IWA 10, BW 348-1, NERICA L19, FARO 57 and LADY'S FINGER. At the core level (paired accessions), cluster analysis delineated 10 pairs of groups consisting 20 genotypes representing 80% of the collections studied. Five accesions representing 20% of the collection and of Nigerian origin exhibited reasonable genetic dispersion forming single clusters.

Discussion

Genetic diversity assessment of rice genotypes is a vital component in germplasm characterization and conservation for the potential identification of parents (Sajib et al., 2012). Morphological and seed traits have been used to study taxonomy and variability among plant species (Lewu et al., 2007). Among several other methods, microsatellites are among the most broadly used DNA marker for various purposes which include genetic diversity study, genome mapping and identification of crop varieties (Teixeira da Silva, 2005). Unlike the morphological and biochemical markers, environmental factors and agronomic practices do not have influence on the outcome of molecular markers techniques (Ovesná et al., 2002). Previous workers (Singh et al., 2004; Joshi and Behera, 2006) have reported the use of molecular markers to investigate genotypic variations among different cultivars. The present investigation addresses the utilization of 21 SSR (microsatellite) markers to reveal genetic polymorphism and ensures unambiguous identification of 25 rice accessions consisting of both exotic and Nigerian elite genotypes. The mean alleles (3.67) obtained in the current study is comparable with previous study by Etemad et al., (2012) that reported 3.57 alleles per SSR locus used on 26 accessions of rice which consist of 13 Iranian and 13 Malaysian cultivars. In a different report, Hossain et al., (2012) discovered an average of 3.8 alleles per locus in landraces of Bangladeshi aromatic rice. The results of this study can be compared to 2.0 – 5.5 alleles per SSR locus for several classes of microsatellites described by Cho et al., (2000) in a study using different genotypes of rice germplasms. Similar result was obtained by Mahalingam *et al*., (2013) who found 2.46 average number of alleles per SSR locus. Wong et al., (2009) reported the genetic relationship and diversity among 8 Bario rice cultivars using 12 SSR primers. The stuy detected a total of 31alleles with an average number of 2.6 alleles per locus, which is lower than the result obtained in this study.

The results of Hossain et al., (2007) observed 3 to 9 alleles with an average of 4.53 alleles per locus for 30 microsatellite markers. Similar results was also reported by Siwach et al., (2004) with an average allele of 4.58 per locus for the various classes of microsatellites used. In the present study, the number of alleles detected was lower than the average number of alleles reported in earlier works (Xu et al., 2004; Jain, et al., 2004; Jayamani et al., 2007; Zeng et al.,

2007; Prathepha, 2012) which reported an average of 11.9, 7.8, 14.6, 7.7 and 11.85 alleles per locus using rice genetic resources from USA, India, a diverse collection of Portuguese rice, landraces from China and wild rice (*Oryza rufipogon*) from North-eastern Thailand and Laos respectively.

Markers with the highest number of identifiable alleles could be the best markers for molecular characterization and diversity study. In the present report, the level of polymorphism determined by the PIC value (mean = 0.48) is consistent with the reported PIC value in previous works (Lu, et al., 2005; Wong et al., 2009; Hossain et al., 2012; Sajib et al., 2012). According to early reports, PIC values ranged from as low as 0.24 to a high of 0.92 with an averaged of 0.61 (Jain et al., 2004) and 0.19 to 0.90 range with an average of 0.75 (Borba et al., 2009), which is markedly higher than the result of this study. Mahalingam et al., (2013) and Upadhyay et al., (2011) reported average PIC values of 0.44 and 0.78 respectively. This study revealed that RM 256 with the highest PIC value would be best in screening the 25 rice genotypes followed by RM 304, RM 3 and so on. Thus, PIC value indicates that all these primers were highly informative and capable of distinguishing between genotypes.

The genetic dissimilarity between the 25 rice genotypes was also determined using a dissimilarity matrix. Generally, modern rice cultivars shared a relatively narrow genetic background when compared to the unexplored vast variability existing in rice landraces worldwide. Thus, it is highly essential not to only conserve landrace genotypes, but also to reveal the gene pool of rice landraces in order to unlock valuable genes that could be useful for breeding programmes (Rabbani et al., 2008). As revealed by microsatellite markers in this report, the high range of genotypic similarity values provides better confidence for the assessments of genetic relatedness and diversity which can be employed in future breeding and conservation programmes. Using SSR markers and cluster analysis, diverse distantly related rice genotypes could be combined by intercrossing genotypes to obtain new hybrids with highest heterosis. Significant greater allelic diversity of microsatellite markers have been reported more than other molecular markers (McCouch et al., 2001). A close assessment of the 25 genotypes studied revealed relatively high similarity among accessions from the same origin. For instance, 60% of the accessions from IITA formed paired clusters. Of the 3 accesions from Malaysia, cluster analysis paired 2 genotypes from Malaysia with the third paring with one of the IITA genotypes. Apart from the pair of IRO 7A & IRO 6N (from the same location), Abakaliki formed pair of clusters with other genotypes collected from IITA. Besides the 3 genotypes identified as IITA-Phillipines, it is possible that the genotypes from IITA genebank share same origin with genotypes from Malaysia. In addition, Abakaliki genotypes demonstrated relatively high genetic relationship with IITA collections which could have been the original source of the materials renamed by NARES scientists. It could be concluded that all the genotypes in this study exhibited reasonable level of locational groupings (Lewu et al., 2007).

Conclusion

The results of the diversity studies using microsatellite markers revealed the degree of similarity among the rice genotypes and were clustered into five dictinct classes according UPGMA analysis and that distantly related genotypes may be combined by intercrossing to get hybrid varieties. Also the application of molecular techniques in the study of genetic diversity and characterization would be of great help in identifying the relatedness of rice genotypes and assist in the selection of parents for hybridization purposes. Therefore, increasing the number of

markers would give a clear idear about the genetic variation and diversity which would ultimately be of interest in the development of rice varieties. In addition, the identification of closely related genotypes through the application of molecular technique could assist to reduce high population of genetic materials in medium-term Genebank by transfering these materials into long term preservation. Information on specific genotypes of interest to local and international research communities combined with verse documentation of closely related genotypes can be use to inform conservation strategies.

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Table 1. Name, country of origin and status of accessions included in the study

Accession Name	Country of Origin	Collection Status
MR 220	Malaysia	Cultivar
MR 263	Malaysia	Cultivar
MR 269	Malaysia	Cultivar
FARO 44	IITA Nigeria	Cultivar

FARO 57	IITA Nigeria	Cultivar
NERICA L 19	IITA Nigeria	Cultivar
BM 348-1	IITA Nigeria	Inbred line
WITA 4	IITA Nigeria	Inbred line
NERICA 2	IITA Nigeria	Cultivar
NERICA 6	IITA Nigeria	Cultivar
NERICA 8	IITA Nigeria	Cultivar
OS 6	IITA Nigeria	Inbred line
LAC 23	IITA Nigeria	Inbred line
IWA 8	Abakaliki Nigeria	Landraces
IWA 10	Abakaliki Nigeria	Landraces
IR06N	Abakaliki Nigeria	Inbred line
IR06A 119	Abakaliki Nigeria	Inbred line
IR07A 108	Abakaliki Nigeria	Inbred line
FADAMA 42	Abakaliki Nigeria	Inbred line
LADYSFINGER	Abakaliki Nigeria	Landraces
INPARI 10	Abakaliki Nigeria	Landraces
UPIA	Abakaliki Nigeria	Landraces
IRRI 154	IITA Nigeria (Philippines)	Cultivar
NERICA L 30	IITA Nigeria (Philippines)	Cultivar
NERICA L 34	IITA Nigeria (Philippines)	Cultivar

Table 2: DNA quantification and quality measurement from Nanodrop photometer

Well	Genotypes	Concentration	Units	260/280	20ng/ul	100Vol
1	MR 220	729.1	ng/ul	1.89	3	97
2	MR 263	937. 3	ng/ul	1.95	2	98
3	MR 269	2495	ng/ul	2.01	1	99
4	FARO 44	750.9	ng/ul	1.94	3	97

5	FARO 57	970.7	ng/ul	1.94	2	98
6	NERICA L 19	1203	ng/ul	2	2	98
7	BW 348-1	607.8	ng/ul	1.85	3	97
8	WITA 4	935.9	ng/ul	1.91	2	98
9	NERICA 2	851. 3	ng/ul	1.95	2	98
10	NERICA 6	1300	ng/ul	1.92	2	98
11	NERICA 8	551.9	ng/ul	1.93	4	96
12	OS 6	1083	ng/ul	1.91	2	98
13	LAC 23	728. 3	ng/ul	2	3	97
14	IWA 8	1109	ng/ul	1.95	2	98
15	IWA 10	578.1	ng/ul	1.93	3	97
16	IR06N	618.1	ng/ul	1.83	3	97
17	IR06A 119	3207	ng/ul	1.8	1	99
18	IR07A 108	1009	ng/ul	1.94	2	98
19	FADAMA 42	854.1	ng/ul	1.96	2	98
20	LADYSFINGER	738	ng/ul	1.88	3	97
21	INPARI 10	866.1	ng/ul	1.95	2	98
22	UPIA	697.6	ng/ul	1.82	3	97
23	IRRI 154	3565	ng/ul	1.85	1	99
24	NL 30	533.4	ng/ul	1.87	4	96
25	NERICA L 34	415.1	ng/ul	1.73	5	95

Table 3: Twenty one traits linked microsatellite (SSR) markers used to assess genetic diversity among 25 rice genotypes

PRIMER	FORWARD PRIMER SEQUENCE	REVERSE PRIMER SEQUENCE
RM 163	TCTAGGGTTAGGGTTTCGCC	AGGTCGGTTCCTTTTGTCC
RM 510	AACCGGATTAGTTTCTCGCC	TGAGGACGACGAGCAGATTC
RM 29	CAGGGACCCACCTGTCATAC	AACGTTGGTCATATCGGTGG

RM 224	ATCGATCGATCTTCACGAGG	TGCTATAAAAGGCATTCGGG
RM 229	CACTCACACGAACGACTGAC	CGCAGGTTCTTGTGAAATGT
RM 247	TAGTCCGATCGATGTAACG	CATATGGTTTTGACAAAGCG
RM 256	GACAGGGAGTGATTGAAGGC	GTTGATTTCGCCAAGGGC
RM 277	CGGTCAAATCATCACCTGAC	CAAGGCTTGCAAGGGAAG
RM 413	GGCGATTCTTGGATGAAGAG	TCCCCACCAATCTTGTCTTC
RM 3	ACACTGTAGCGGCCACTG	CCTCCACTGCTCCACATCTT
RM 276	CTCAACGTTGACACCTCGTG	TCCTCCATCGAGCAGTATCA
RM 590	CATCTCCGCTCTCCATGC	GGAGTTGGGGTCTTGTTCG
RM 219	CGTCGGATGATGTAAAGCCT	CATATCGGCATTCGCCTG
RM 8	CACGTGGCGTAAATAVACGT	GGCCAAACCCTAACCCTG
RM 288	CCGGTCAGTTCAAGCTCTG	ACGTACGGACGTGACGAC
RM 443	GATGGTTTTCATCGGCTACG	AGTCCCAGAATGTCGTTTCG
RM 304	TCAAACCGGCACATATAAGAC	GATAGGGAGCTGAAGGAGATG
RM 226	AGCTAAGGTCTGGGAGAAACC	AAGTAGGATGGGGCACAAGCTC
RM 88	ACTCATCAGCATGGCCTTGCTC	TAATGCTCCACCTTCACCAC
RM 317	CATACTTACCAGTTCACCGCC	CTGGAGAGTGTCAGCTAGTTGA
RM 349	TTGCCATTCGCGTGGAGGCG	CTCCATCATCCCTATGGTCG
RM 341	CAAGAAACCTCAATCCGAGC	CTCCTCCCGATCCCAATC

Table 4: Major allele frequency, number of alleles, gene diversity and PIC value of SSR markers

PRIMER	Main Allele Freq.	Allele No	Gene Diversity	Polymorphic Information Content (PIC)
RM163	0.579	4	0.53	0.410
RM510	0.579	4	0.462	0.400
RM29	0.680	4	0.478	0.391
RM224	0.587	3	0.453	0.424
RM229	0.693	3	0.48	0.416
RM247	0.596	5	0.489	0.411
RM256	0.629	3	0.541	0.503
RM277	0.606	4	0.425	0.404
RM413	0.589	3	0.381	0.455
RM3	0.613	3	0.546	0.465
RM276	0.568	3	0.377	0.424
RM590	0.552	4	0.504	0.369
RM219	0.545	3	0.511	0.447
RM8	0.602	4	0.5	0.365
RM288	0.659	5	0.395	0.415
RM443	0.661	4	0.525	0.426
RM304	0.621	4	0.511	0.465
RM226	0.632	4	0.52	0.445
RM88	0.629	3	0.461	0.372
RM 349	0.554	3	0.552	0.446
RM341	0.634	4	0.456	0.413
MEAN	0.610	3.67	0.481	0.422

Table 5: Pairwise genetic distance indices among 25 lowland and upland rice obtained from SSR marker analysis

GENOTYPES	BW348-1	FADAMA42	FARO44	FARO57	INPARI10	IR06A	IR06N	IR07A	IRRI154	IWA10	IWA8	LAC23	LADYSFINGER	MR220	MR263	MR269	NERICA2	NERICA6	NERICA8	NERICAL19	NERICAL30	NERICAL34	OS6	UPIA	WITA4
BW348-1	0.00																								
FADAMA42	0.67	0.00																							
FARO44	0.48	0.67	0.00																						
FARO57	0.29	0.71	0.33	0.00																					
INPARI10	0.48	0.62	0.33	0.33	0.00																				
IR06A	0.48	0.57	0.48	0.48	0.43	0.00																			
IR06N	0.48	0.62	0.43	0.52	0.52	0.33	0.00																		
IR07A	0.57	0.57	0.52	0.62	0.57	0.38	0.29	0.00																	
IRRI154	0.57	0.57	0.43	0.43	0.38	0.43	0.48	0.48	0.00																
IWA10	0.24	0.57	0.38	0.33	0.43	0.33	0.43	0.52	0.43	0.00															
IWA8	0.52	0.62	0.48	0.43	0.48	0.33	0.38	0.43	0.29	0.29	0.00														
LAC23	0.48	0.62	0.57	0.57	0.62	0.62	0.52	0.67	0.71	0.62	0.71	0.00													
LADYSFINGER	0.38	0.62	0.29	0.14	0.29	0.43	0.48	0.52	0.38	0.33	0.43	0.62	0.00												
MR220	0.57	0.71	0.43	0.57	0.62	0.52	0.48	0.52	0.62	0.43	0.43	0.67	0.57	0.00											
MR263	0.57	0.67	0.48	0.48	0.52	0.43	0.48	0.52	0.57	0.43	0.33	0.76	0.48	0.29	0.00										
MR269	0.57	0.67	0.29	0.38	0.48	0.48	0.43	0.52	0.38	0.52	0.38	0.71	0.38	0.43	0.38	0.00									
NERICA2	0.48	0.48	0.67	0.62	0.67	0.62	0.67	0.76	0.62	0.57	0.57	0.33	0.67	0.76	0.76	0.71	0.00								
NERICA6	0.43	0.57	0.62	0.48	0.67	0.57	0.48	0.57	0.71	0.57	0.62	0.24	0.57	0.67	0.62	0.67	0.43	0.00							
NERICA8	0.43	0.57	0.48	0.48	0.57	0.57	0.52	0.67	0.67	0.57	0.62	0.19	0.57	0.67	0.62	0.57	0.29	0.19	0.00						
NERICAL19	0.33	0.67	0.29	0.05	0.29	0.43	0.48	0.57	0.38	0.29	0.38	0.62	0.10	0.57	0.43	0.33	0.67	0.52	0.52	0.00					
NERICAL30	0.43	0.57	0.57	0.38	0.48	0.38	0.52	0.52	0.29	0.29	0.24	0.62	0.43	0.52	0.52	0.52	0.52	0.62	0.57	0.43	0.00				
NERICAL34	0.62	0.71	0.48	0.48	0.19	0.38	0.48	0.52	0.29	0.43	0.33	0.67	0.43	0.57	0.48	0.52	0.71	0.71	0.62	0.43	0.33	0.00			
OS6	0.52	0.62	0.52	0.57	0.57	0.62	0.62	0.71	0.71	0.67	0.71	0.43	0.62	0.67	0.71	0.62	0.38	0.38	0.38	0.62	0.71	0.71	0.00		
UPIA	0.57	0.43	0.29	0.43	0.38	0.43	0.48	0.48	0.24	0.43	0.33	0.62	0.33	0.57	0.48	0.38	0.57	0.62	0.57	0.38	0.38	0.43	0.71	0.00	
WITA4	0.52	0.76	0.43	0.38	0.24	0.48	0.48	0.57	0.38	0.48	0.43	0.71	0.43	0.57	0.38	0.43	0.76	0.67	0.62	0.33	0.52	0.24	0.62	0.57	0.00

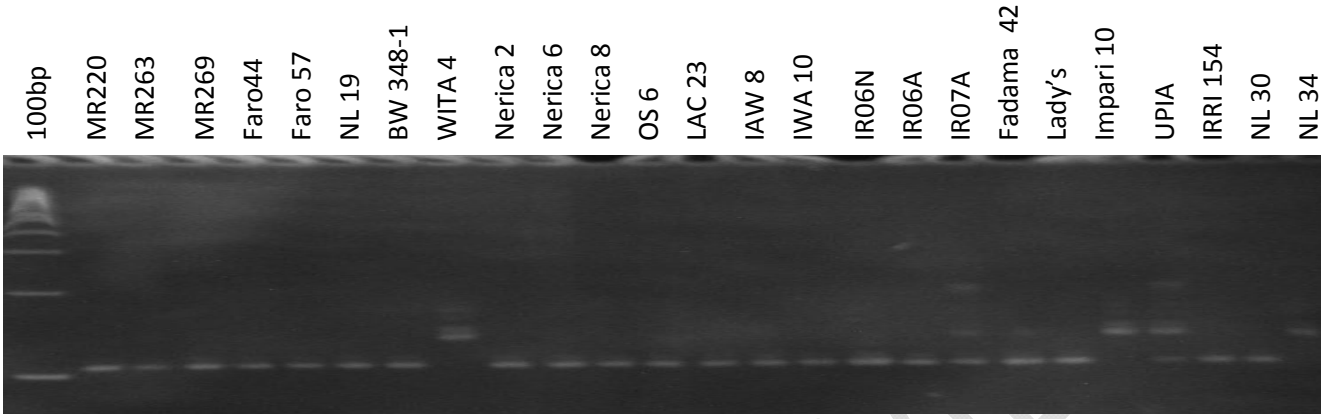

Figure 1: RM 256

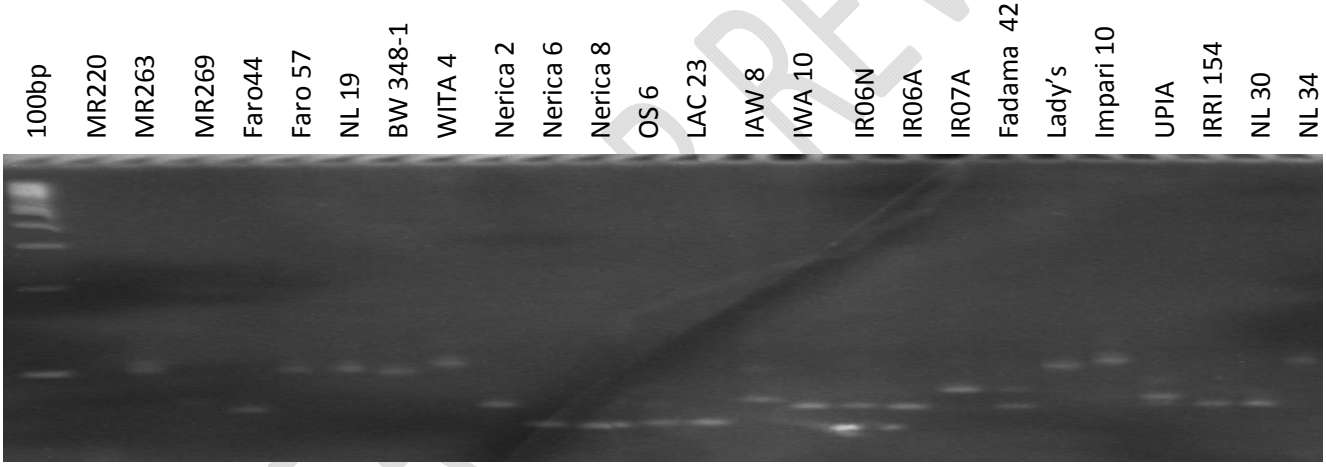

Figure 2: RM 413

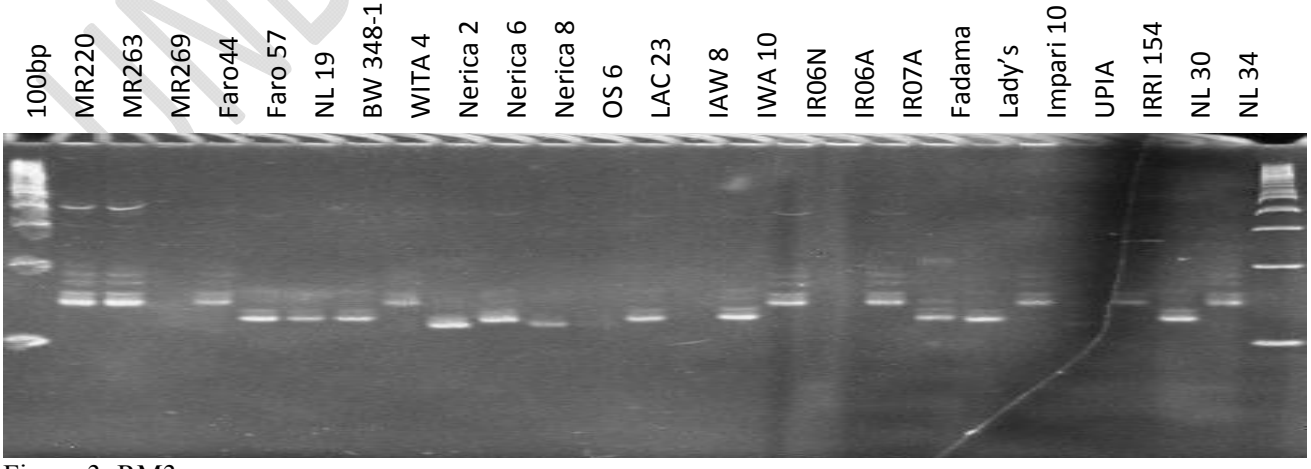

Figure 3: RM3

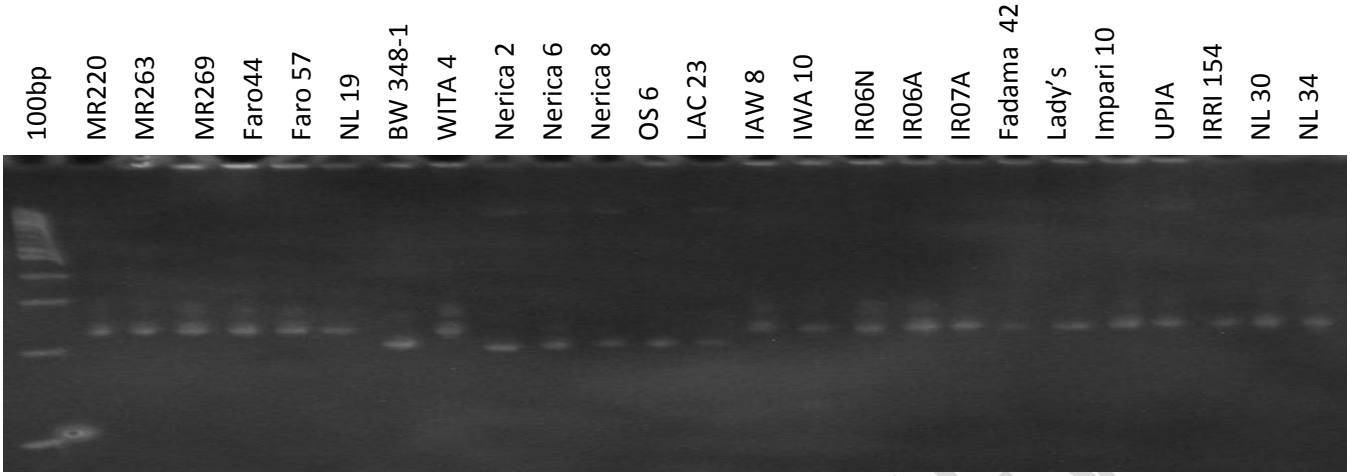

Figure 4: RM 219

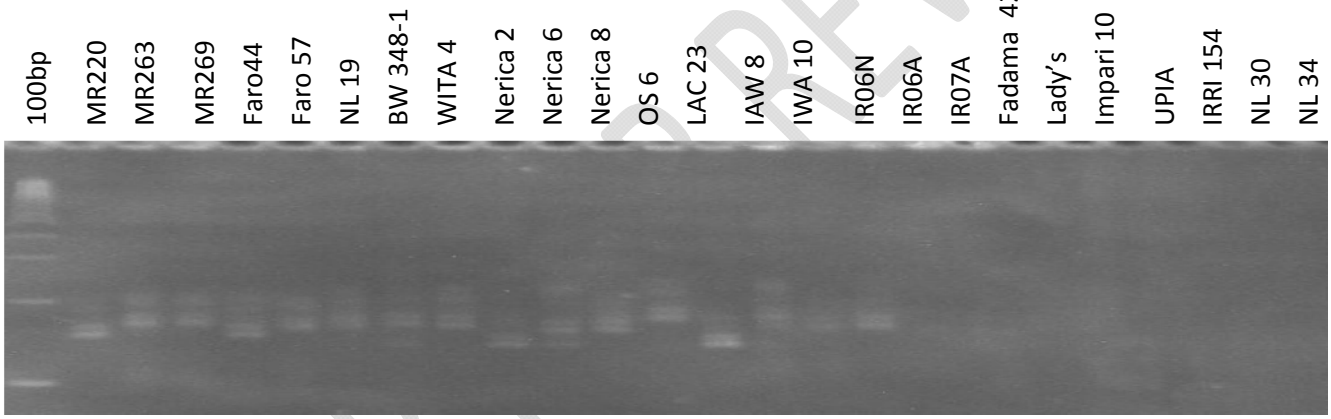

Figure 5: RM304

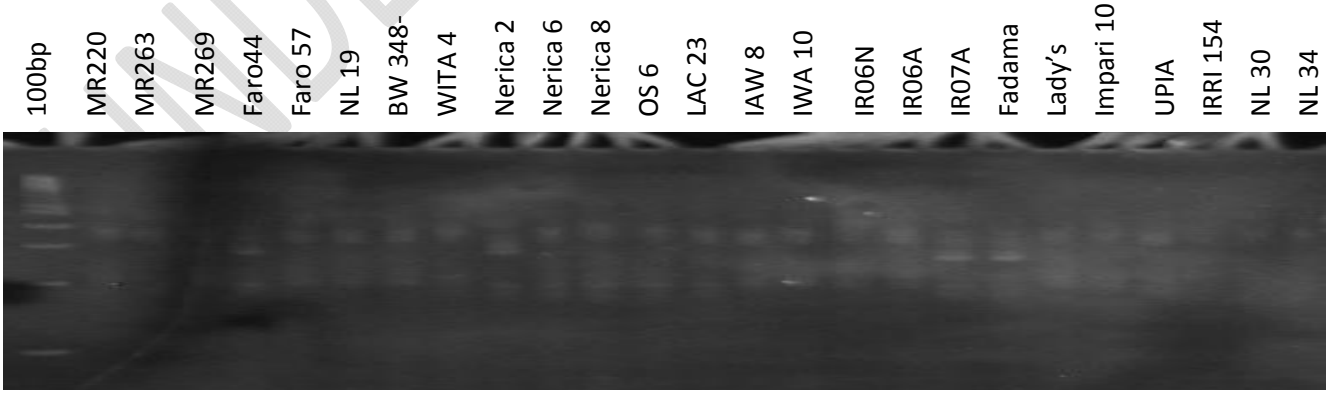

Figure 6: RM226

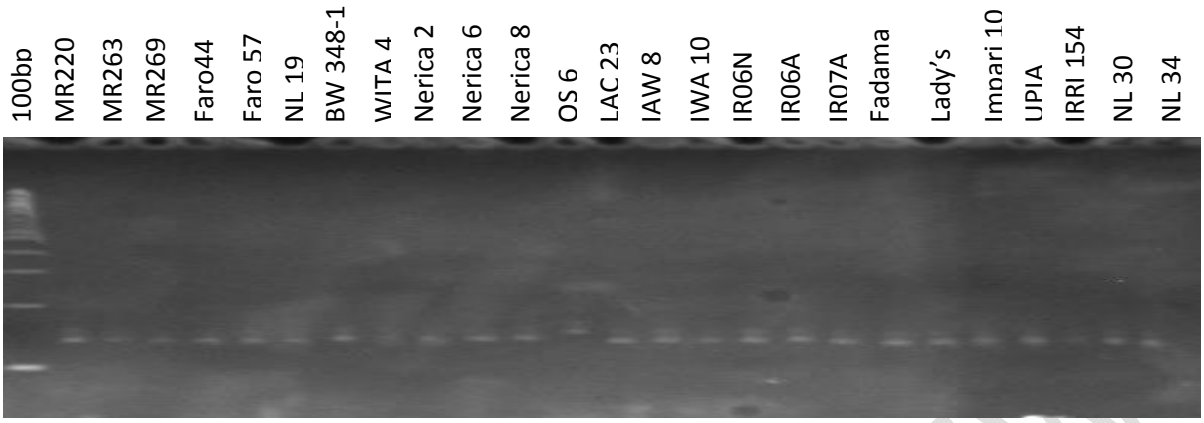

Figure 7: RM 349

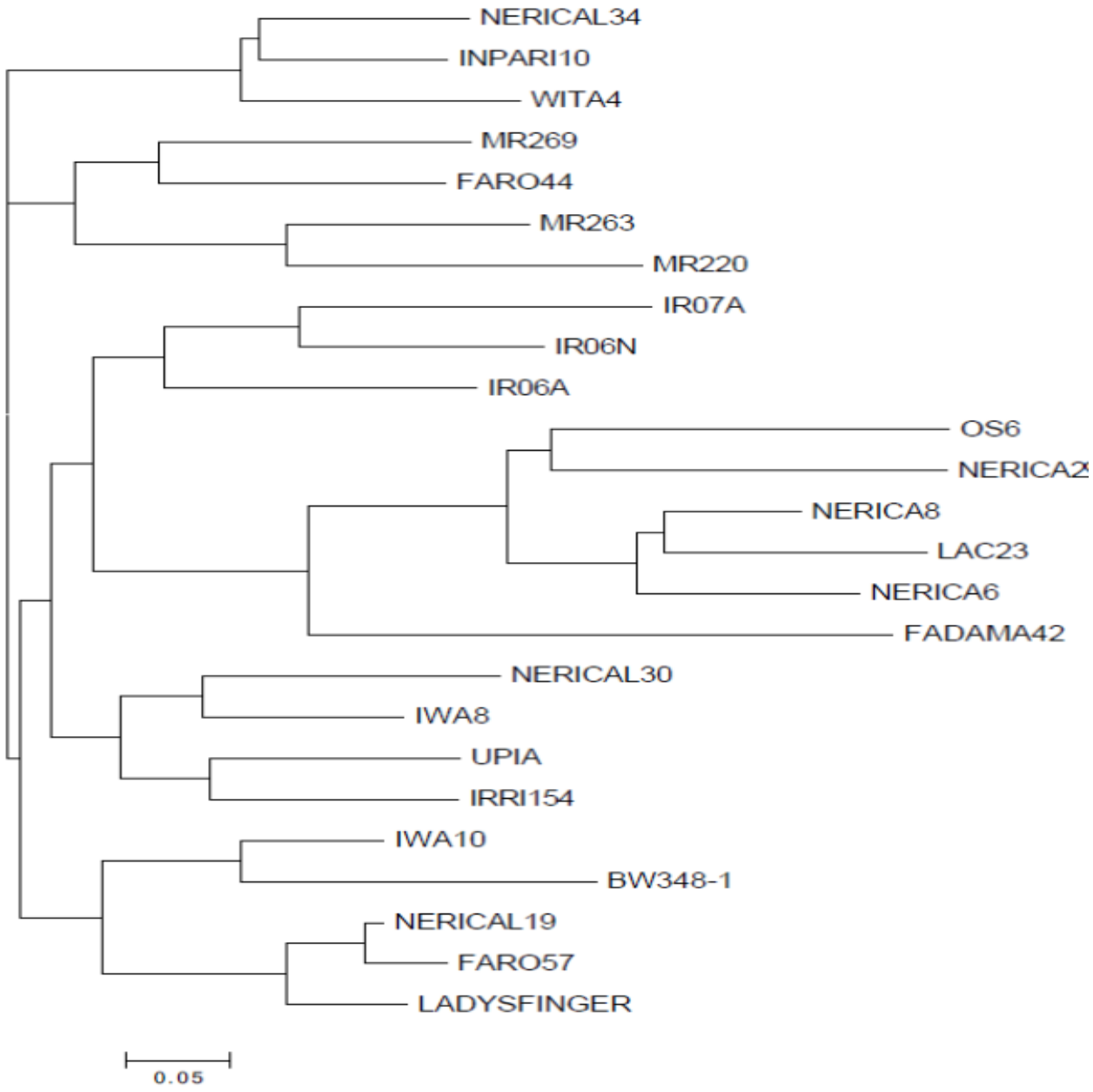

Figure 8: An unrooted neighbor-joining tree showing the genetic relationships among exotic and Nigerian elite genotypes obtained from SSR marker analysis.