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Allelic data showing interconnectedness in rice species (*Oryza sativa, Oryza glaberrima, Oryza barthii*) and the interspecific hybrids (NERICA)

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The current level of genome coverage provided by microsatellite markers in rice is sufficient for DNA fingerprinting, providing Information on diversity and population structure which is expected to assist plant breeders by providing a more rational basis for expanding the gene pool and for identifying material that harbours alleles of value for plant improvement. Allelic data from the species studied revealed that rice interspecific hybrids (NERICA) were more closely related to *Oryza sativa* than to *Oryza glaberrima* which was closely associated to *Oryza barthii* than to *O sativa*. Comparative polymorphism between species showed that *O. sativa, Nerica, Oryza barthii* and *O. glaberrima* produced 51, 45, 40 and 36 alleles with 1461, 238, 305 and 445 polymorphic bands respectively with 17 SSR markers. Alleles ranged from 2 to 4 in *O. sativa* and nerica; from 2 to 3 in *O. glaberrima* and 1 to 4 in *O. barthii*. The average polymorphic information content and resolving power was highest in *O. sativa* (0.53, 0.63), followed by nerica (0.52, 0.62), next was *O. barthi* (0.29, 0.30) and least in *O. glaberrima* (0.22, 0.22). Alleles at RM508 were most reliable in profiling *O. barthii* genotypes. Definitive markers (RM240, RM488) for *O. glaberrima* and *O. sativa* (RM587) were obtained. Cluster analysis grouped the rice genotypes into ten clusters at a similar coefficient of 68% with genotypes of the same genetic similarity clustering together. Based on this study therefore, molecular fingerprinting and crop improvement could be advanced for breeding programmes.

Key words: Nerica, *Oryza barthii, Oryza glaberrima, Oryza Sativa,* rare allele.

INTRODUCTION

Genomic relationship among Oryza, an agronomically important genus with basic chromosome number of twelve has been delineated by various kinds of genetic and cytogenetic analysis including chromosome pairing (Katayama, 1982), morphology (Morishma and Oka, 1970), isozymes (Glaszman, 1987) and DNA polymorphism (Ren et al., 2003). *Oryza sativa* and *Oryza glaberrima* are AA genome species with minor sub

genomic differences that evolved by independent and parallel evolutionary process in Asia and Africa (Ghesquiere et al., 1977). The knowledge of the extent and structure of genetic diversities and species relationships in the genus Oryza is essential not only for understanding the process of evolution but also for the development of appropriate and efficient strategies for the collection, conservation and introgression of useful genes to cultivated crops.

Our hypothesis is that the African rice gene pool is still underutilized. Therefore, the objectives of this study were to evaluate the genetic diversity of rice genotypes based

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on microsatellite markers and to determine differences in pattern of diversity among and within species. Information was also provided about rare alleles that can be used in cultivar advancement.

MATERIALS AND METHODS

One hundred and fifty (150) viable seeds of each rice genotype (comprising of 92 *O. sativa* species of Asia and African origin; 27 *O. glaberrima*, 16 *O. barthi* species and 15 interspecific hybrid (*O. sativa × O. glaberrima*) (Table 1) were obtained from the International Rice Research Institute, Las Boanos and the African Rice Center (AfricaRice) Ibadan station, Nigeria.

DNA extraction and SSR markers

Genomic DNA was extracted from young leaf tissues obtained from the sreenhouse following the method described by Dellaporta et al. (1982), using 5 mg of leaf tissue from at least three seedlings. Thirty two microsatellite markers were evaluated of which Seventeen (17) polymorphic rice microsatellite markers were obtained. The original sources and motifs for these markers were found in Temnykh et al. (2000) and rice gene database (http:llars.genome.cornell.edu/rice) verified in October 2, 2001). The nucleic acid concentration was quantified using ND-1000 spectrophotometer (Nano- Drop Technologies inc. 2007. www. Nanodrop.com)

Polymerase chain reaction

Polymerase chain reaction was run using 10 μl reaction mixture containing 1 μl 10* PCR buffer, 0.5 μl of 25 m MgCl2, 0.2 μl of 10 mM DNTP's, 0.25 μl of 20 pm primer pair, 0.1 μl of Taq polymerase, 2.10 μl milu-Q water and 5 μl of l0 ng/μl DNA. The reaction was run using Perkin- Elmer thermocycler according to the following amplification procedure: 2 min pre heating at 94°C, followed by 34 cycles of 30 s denaturing at 94°C, 30 s annealing at 55 and 67°C depending on the primer pair used, 30 s initial extension time at 72°C and final extension of 2 min at 72°C. The amplified products were subjected to electrophoresis using a 2% agarose gel in 1* TBE buffer at 78 to 80 volts for 2 to 3 h for good band separation. The gel was stained with 0.5 μl/L ethidium bromide for 5 min and visualized under ultra- violet light using a gel documentation system.

Data analysis

Polymorphic information content (PIC)

The value of a marker for detecting polymorphism (Polymorphic Information Content (PIC) between genotype for each primer combination depending on the number of detectable alleles and the distribution of their frequency was calculated following the formula proposed by Anderson et al. (1993).

$$PIC_i = 1 - \sum_{J-1}^{N} P_{ij}^2$$

where P_{ij} is the frequency of the jth allele for marker 'i' and summation extends over n alleles.

Band Informativeness: I_b

The ability of the primer to distinguish between accessions was assessed by calculating their resolving power (Rp) as $Rp = \sum I_b$ where I_b is band informativeness, $I_b = 1 - [2 \times (0.5 - P_i)]$ and P_i is the proportion of accession containing band I (Prevost and Wilkinson, 1999).

Cluster analysis

The number of repeats for each allele was determined by comparing the size of the PCR products with that of IR36 whose repeat number was characterized by Temnykh et al. (2000). The estimated repeats amplified fragments was treated as a unit character and scored as a binary code 1 and 0 for presence and absence respectively. Only prominent and unambiguous bands were scored. Genetic similarities were evaluated using Jaccard similarity coefficient for pair-wise comparisons based on the proportion of shared bands produced by primers generated using 'SMQUAL' sub-program of NTSYS-pc software (Rohlf, 2000).

RESULTS

Comparative polymorphism between species

SSR Polymorphism showed that *O. sativa* genotypes produced 51 allele and a total of 1461 bands with an average allele per marker of 3, ranging from 2 to 4 (Table 1). Forty five (45) alleles were detected in interspecific hybrids with the number of alleles per marker ranging from 2 to 4 with an average of 2.65 (Table 2). In *O. glaberrima* genotypes, 36 alleles were obtained with 445 bands and an average allele per marker of 2.12 ranging from 1 to 3 (Table 3). *O. barthii* had a monomorphic allele at RM 216 with alleles per marker ranging from 1 to 4 and produced a total of 40 alleles with 305 bands with an average allele of 2.4 (Table 4).

The number and average number of alleles obtained was highest in *O. sativa* where four markers revealed 4 alleles each. The average number of alleles for the interspecific hybrids and *O. glaberrima* were 2.65 and 2.12, respectively.

O. sativa did not share an allele at RM587 and RM240 with other species. These markers could serve in fingerprinting *O. sativa* genotypes. However, *O. sativa* shared 5 alleles with the interspecific hybrids (two at RM501 and one each at RM 508, RM 520 and RM 539). The interspecific hybrids did not share 13.3% alleles with *O.glaberima*; 6.67% with *O. sativa* and 8.8% with *O. barthii*. The interspecific hybrids and *O. glaberrima* had one alleles absent at the same locus at RM84, RM240, RM286, RM508 and RM587. With *O. barthii*, the interspecific did not share the same allele at the same locus at RM1, RM240 and RM587. In *O. glaberrima* 38.8, 33.3 and 19.4% of its alleles were absent in *O. sativa*, Interspecific hybrids and *O. barthii* respectively. With *O. barthii*, 22.5% of its allele were absent in *O. sativa* and in the interspecific hybrids, while only 7.5% alleles were not detected in *O. glaberrima*. *O. barthii* exclusively possessed

Table 1. Data on number of alleles, polymorphic information content (PIC) value, band informativeness, resolving power and allele frequency of 17 SSR markers for 92 *O. sativa* genotypes.

Marker	Allele	Rare allele	PIC	IB	Allele frequency
RM1	3	0	0.62	0.76	95.65
RM17	2	0	0.20	0.21	97.83
RM21	3	0	0.70	0.91	100.00
RM84	3	4	0.19	0.20	98.91
RM216	2	0	0.50	0.59	96.74
RM240	4	0	0.27	0.29	94.57
RM286	4	1	0.66	0.83	94.57
RM483	3	2	0.50	0.59	92.39
RM488	3	0	0.59	0.72	94.57
RM495	2	0	0.44	0.50	100.00
RM501	4	0	0.62	0.76	88.04
RM508	3	0	0.43	0.49	91.30
RM518	2	0	0.51	0.60	95.65
RM520	3	0	0.70	0.90	91.30
RM539	3	0	0.75	1.00	80.43
RM587	4	1	0.83	0.82	93.48
RM589	3	0	0.54	0.62	94.05
Average	3		0.53	0.63	91.75

Table 2. Data on number of alleles, PIC value, resolving power and allele frequency of 17 SSR markers for 15 Interspecific hybrids (NERICA).

Marker	Allele	Rare allele	PIC	IB	Allele frequency
RM1	3	1	0.63	0.78	93.33
RM17	2	0	0.48	0.56	100.00
RM21	3	0	0.59	0.72	93.33
RM84	2	1	0.13	0.14	100.00
RM216	2	0	0.45	0.52	93.33
RM240	3	0	0.41	0.46	100.00
RM286	3	1	0.57	0.69	93.33
RM483	2	0	0.54	0.64	93.33
RM488	2	0	0.39	0.36	86.67
RM495	2	0	0.29	0.31	100.00
RM501	4	0	0.59	0.72	80.00
RM508	3	0	0.65	0.82	86.67
RM518	2	0	0.23	0.25	100.00
RM520	3	1	0.52	0.61	86.67
RM539	3	1	0.71	0.92	80.00
RM587	3	0	0.58	0.70	93.33
RM589	3	0	0.62	0.76	92.52
Average	2.65		0.52	0.62	92.50

Table 3. Data on number of alleles, PIC value, resolving power and allele frequency of 17 SSR markers for 27 *O. glaberrima* genotypes.

Marker	Allele	Rare allele	PIC	IB	Allele frequency
RM1	2	0	0.1	0.1	100.00
RM17	2	1	0.07	0.08	100.00
RM21	2	0	0.38	0.43	100.00

Table 3. Contd.

RM84	2	0	0.13	0.14	100.00
RM216	1	0	0	0	100.00
RM240	3	0	0.55	0.66	100.00
RM286	2	1	0.14	0.15	96.30
RM483	2	0	0.32	0.35	96.30
RM488	3	0	0.63	0.78	96.30
RM495	3	2	0.07	0.08	100.00
RM501	2	0	0.16	0.17	88.89
RM508	2	0	0.25	0.27	100.00
RM518	2	0	0.2	0.21	100.00
RM520	2	0	0.13	0.14	100.00
RM539	2	0	0.31	0.34	100.00
RM587	2	0	0.13	0.14	100.00
RM589	2	0	0.13	0.14	100.00
Average	2.12		0.22	0.25	98.69

Table 4. Data on number of alleles, PIC value, resolving power and allele frequency of 17 SSR markers for 16 *O. barthii* genotypes.

Marker	Allele	PIC	IB	Allele frequency
RM1	2	0.08	0.08	100
RM17	2	0.39	0.44	100
RM21	3	0.22	0.24	100
RM84	3	0.22	0.24	100
RM216	1	0	0	100
RM240	2	0.23	0.25	100
RM286	4	0.26	0.24	100
RM483	2	0.02	0.02	93.75
RM488	3	0.6	0.74	100
RM495	3	0.65	0.57	100
RM501	2	0.66	0.58	100
RM508	3	0.19	0.2	100
RM518	2	0.49	0.57	100
RM520	2	0.3	0.33	100
RM539	2	0.21	0.22	100
RM587	2	0.21	0.22	100
RM589	2	0.21	0.22	100
Average	0.24	0.29	0.3	99.63

an allele at RM 508 which was absent in the other species.

Allele frequency

The average allele frequency for *O. sativa, O. glaberrima, O. barthii* and the interspecific hybrids was 91.75, 98.6, 99.63 and 92.50% respectively. *O. sativa* had the least mean allelic frequency of 91.75%, while *O. barthii* had the highest mean allelic frequency of 99.63% followed by the

interspecific hybrid with 92.50% (Tables 1 to 4).

Rare alleles

Eight rare alleles were detected in *O. sativa* genotypes with the highest number of four at RM84. RM483 marker produced two rare alleles while RM 240 and RM 587 revealed one rare allele each. Two genotypes (ITA235 and ITA 150) were tagged as possessing rare alleles with RM587 and RM286 respectively. At RM482, two genotypes (IR42 and LAC 23) with rare alleles were also tagged. While at RM84 four genotypes with rare alleles were detected. Rare alleles were consistently detected with RM 286. *O. glaberrima* had 11.1% rare alleles tagged at RM 495, RM 17 and RM 286. Rare alleles were not detected for *O. barthii* and the interspecific hybrids. (Tables 1 to 4).

PIC value and resolving power (IB)

The PIC values revealed that *O. sativa* and the interspecifics had higher values than that obtained in *O. glaberrima* and *O. barthii*. The average PIC value was 0.53, 0.52, 0.22 and 0.29 for *O. sativa*, interspecifics, *O. glaberrima* and *O. barthii* respectively. Informative primers (PIC>0.5) were obtained in all species evaluated. (Tables 1 to 4). The resolving power of the primers varied between species and was highest in *O. sativa* (0.63) and lowest in *O. glaberrima* (0.25). *O. barthii* had an average Rp of 0.3. (Tables 1 to 4).

Clustering of rice genotypes

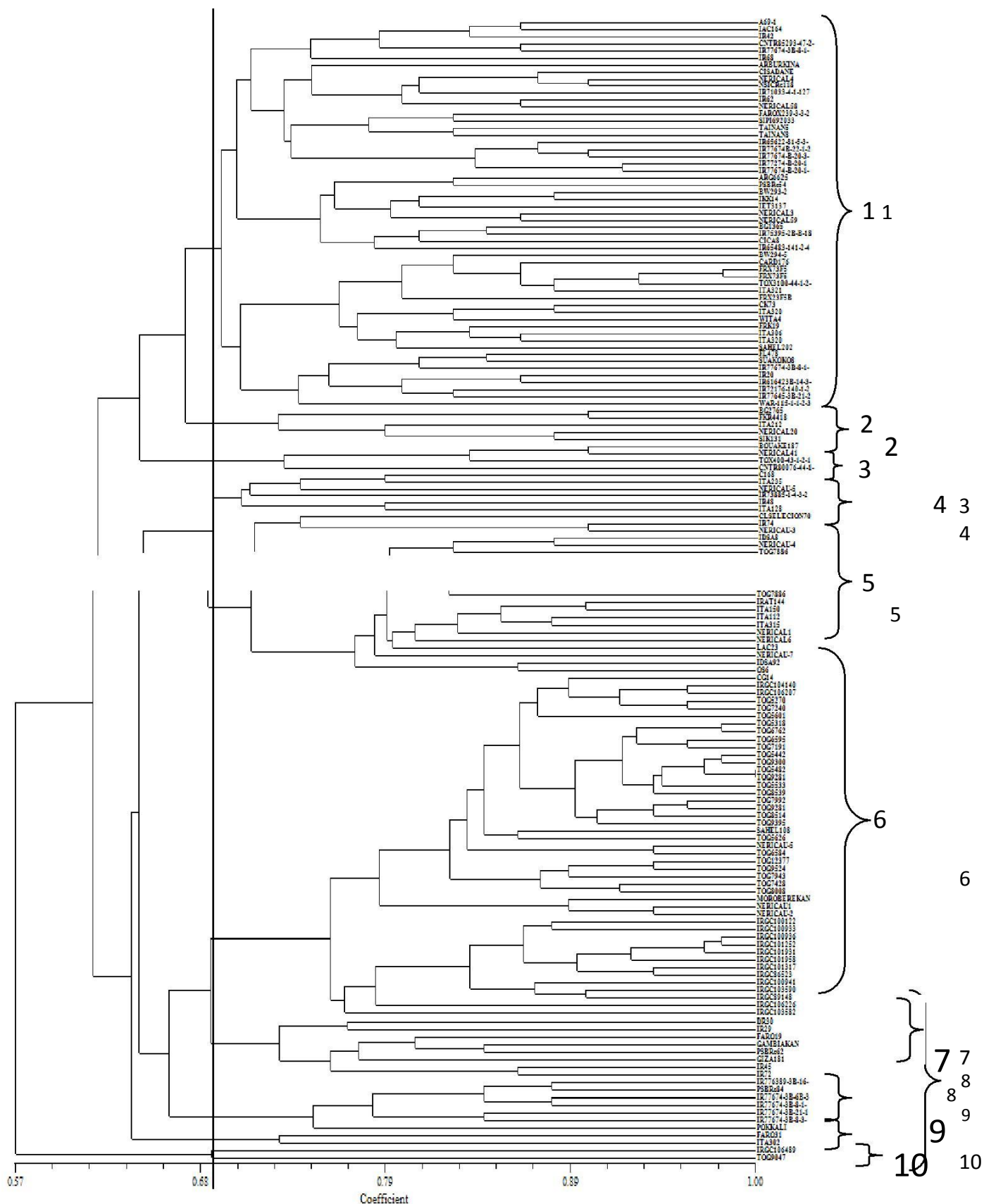The dendrogram (Figure 1) at 68.5% similarity coefficient

Figure 1. UPGMA dendrogram showing the genetic diversity of 150 rice genotypes based on 17 polymorphic SSR markers. Cluster Group 1- African and Asian *O. sativa* and Interspecifics (A 69-1, IAC 164, IR 42, CNTR 85293-47-2-1-1,

IR 77674-3B-8-1-3-4-5, IR 68,AR BURKINA,CISADANE, NERICA L – 4, NSIC Rc 118, IR 71033-4-1-127, IR 62, NERICA L-58, FAROX 239-3-3-2, SIPI 692033, TAINAN 5, TAINAN 8, IR 65622-81-5-3-2, IR 77674 B-22-1-2-1-3-8-B, IR 77674-B-20-3-3-1-3-13-B, IR 77274-B-20-1-2-1-3-6-B, IR 77674-B-20-1-2-1-3-6-B, ARG 6625, PSB Rc 54, BW 293-2, IKK 14, IET 3137, NERICA L-3, NERICA L-59, BG 1365, IR 75395-2B-B-18-1-1-1-11-2, CICA 8, IR 65483-141-2-4-4-2-5' BW 294-5, CARD 176, FRX 73F5-22F6BF8, FRX 73F5-22F6BF8, TOX 3100-44-1-2-3-3, ITA 321, FRX 23F5B-13F6BF8, CK 73, ITA 320, WITA 4, FRK 19, ITA 306, SAHEL 202, FL 478, SUAKOKO8, IR 77674-3B-8-1-1-1-B, IR 20, IR 61642 3B-14-3-3-2, IR 72176-140-1-2, IR 77645-3B-21-23-14-5, WAR-115-1-1-2-3-B-B). Cluster Group 2- African and Asian *O. sativa,* and intraspecifics (BG 2765, FKR (4418 X IR 6115-1-1-1), ITA 212, NERICA L-20, SIK 131). Cluster Group 3- African *O. Sativa* Intraspecifics and Asian *O. Sativa* Outcluster (BOUAKE 187, NERICA L-41, TOX 400-43-1-2-1, CNTR 800 76-44-1-1). Cluster Group 4- upland *O. sativa, intraspecifics* and interspecifics (C 168, ITA 235, NERICA U-5, IR 73885-1-4-3-2-1-6, IR 48, ITA 128). Cluster Group 5- African O. sativa andinterspecifics (CL SELECION 70, IR 74, NERICA U-3, IDSA 8, NERICA U-4, TOG 7886, IRAT 144, ITA 150, ITA 112, ITA315, NERICA L – 1, NERICA L – 6, LAC 23, NERICA U-7, IDSA 92, OS 6). Cluster Group 6- *O. Glaberrima, O. Barthii*, African *O. sativa* and interspecifics (CG 14, IRGC 104140, IRGC 106207, TOG 5270, TOG 7240, TOG 5601, TOG 5318, TOG 6762, TOG 6595, TOG 7191, TOG 5442, TOG 9300, TOG 5482, TOG 9281, TOG 5533, TOG 8539, TOG 7992, TOG 9281, TOG 8514, TOG 9395, SAHEL 108, TOG 5626, NERICA U-5, TOG 6584, TOG 12377, TOG 9524, TOG 7943, TOG 7428, TOG 8008, OROBEREKAN, NERICA U-1, NERICA U-2, IRGC 100122, IRGC 100933, IRGC 100936, IRGC 101252, IRGC 101931, IRGC 101958, IRGC 101317, IRGC 86523, IRGC 100941, IRGC 103590, IRGC 89148, IRGC 106226, IRGC 103582). Cluster Group 7- African and Asian *O. sativa* (DR 30, IR 29, FARO 19, GAMBIAKA (NIGERIA), PSB Rc 62, GIZA 181, IR 45, IR 72). Cluster Group 8- Interspecifics and Asian *O. sativa*(IR 776389-3B-16-2-2-2-1-2, PSB Rc 84, IR 77674-3B-6B-3-3-2-B, IR 77674-3B-8-1-1-10-4, IR 77674-3B-21-1-1-1-2, IR 77674-3B-8-3-1-1-5, POKKALI). *C*luster Group 9- African *O. sativa* (FARO 31, ITA 302). Cluster Group 10- *O. glaberrima and O. barthii.* (IRGC 106489, TOG 9047).

identified 10 groups. Genetically similar genotypes clustered together. Group 1 contained 55 genotypes (51, *O. sativa* and 4 interspecific hybrid). Group 2 comprised of two sub-groups. The first sub-group clustered 2 Asian *O. sativa* genotypes while the second subgroup clustered two African *O. sativa* genotypes and one lowland interspecific hybrid. Group 3 contained two African *O. sativa*; one interspecific hybrid and one Asian *O. sativa* outcluster. Group 4 comprised of six genotypes: One upland interspecific hybrid, two *sativa* and three *O. sativa* genotypes of Asian and African origin respectively. Group 5 were all genotypes of African origin and contained 2 major sub-groups. Group 6, composed of 45 genotypes, and had two major subgroups (similarity coefficient 76.5%). The first subgroup had 2 minor subgroups, with the first subgroup having 29 genotypes. These genotypes were mostly African *O. glaberrima* genotypes (25), 2, *O. barthii*, an upland interspecific hybrid and one *O. sativa* cultivar. The second minor subgroup consisted of two upland interspecific hybrids and an African *O. sativa*. The second major subgroup comprised of 13 *O. barthii* genotypes. Groups 7 and 8 comprised of 8 and 7 genotypes respectively. Genotypes in group 7 were all *O. sativa* of Asian origin. Group 8 comprised of five *O. sativa* intraspecific hybrids and two Asian *O. sativa* genotypes. Group 9 comprised of two African *O. sativa*, while group 10 with just two genotypes at a similar level of 68.5% comprised of one *O. barthii* and one *O. glaberrima.*

DISCUSSION

Simple sequence repeat (SSR) markers are PCR based markers that can detect a significantly higher degree of polymorphism in rice (Yang et al., 1994) and are suitable for evaluating genetic diversity among closely related rice cultivars (Akagi et al., 1997). In rice, molecular markers have been used to identify accessions (Olufowote et al., 1997), determine genetic structure and pattern of diversity for cultivars of interest (Akagi et al., 1997), and optimize assembly of core collection (Schoen and Brown, 1993). The higher number of alleles observed in *O. sativa* could result from the marker specificity or might be attributed to natural and human selection, leading to high genetic diversity of cultigens (Semon et al., 2005). The relatively low genetic variation of the cultivated *O. glaberrima*, compared to *O. barthii* was also noted by other studies using RFLP (Wang et al., 1992), AFLP (Aggarwal et al., 1999) and SSR (Joshi et al., 2000) analysis that the wider geographical distribution of the Asian taxa compared to others may have contributed to the higher genetic variation (Vaughan, 1994). Selection after domestication has led to the immense diversity in varieties that characterizes many domesticated plant species which as Darwin pointed out can exceed the range of phenotypic variation in their wild ancestors (Rindos, 1984). The presence of low genetic variation in species as observed between *O. glaberrima and O. barthii* implies that these species may have undergone a relatively recent differentiation (Vaughan, 1994).

Specie relatedness as depicted by shared alleles indicated the trend of evolution and progenitors of the species. Following this trend therefore, *O. glaberrima* was most closely related to *O. barthii.* Porterez (1970) and Oka (1988) reported that *O. barthii* is a progenitor of *O. glaberrima*. The alleles shared between the interspecifics and *O. sativa* also indicated that the interspecifics might be closer to *O. sativa* than to *O.glaberrima* . The larger number of alleles shared between the interspecifics and *O. sativa* than between the interspecifics and O. glaberrima could be attributed to gene silencing during molecular analysis or that the alleles were lost (Pham and Bougerol, 1993).

The average PIC value observed in *O. sativa* genotypes was higher than that present in *O. glaberrima* and *O. barthii*. The low PIC value observed in *O. glaberrima* and *O. barthii* was consistent with report from Semon et al. (2005). There was no association between the PIC value and the number of alleles detected at a polymorphic locus. The observed pattern was consistent with the result obtained by Yang et al. (1994) using only 10 SSR loci in his work but varied with the report of Yu et al. (2003) based on larger sample size. The highest Rp value was also detected in *O. sativa* and lowest in *O. glaberrima*. *O. sativa* had more informative primers than other species studied. Higher informative primers obtained with the interspecifics than *O. glaberrima* was attributed to the interspecifics possessing more *O. sativa* alleles. A positive correlation was observed for PIC and Rp value. The presence of rare alleles seemed positively associated with the number of genotypes studied. Rare alleles generated were larger with *O. sativa* genotypes followed by *O. glaberrima.* The presence of rare alleles in these species indicated that these species may be useful to plant breeders and geneticists as a rich source of genetic material. Markers associated with rare alleles could also be utilized in marker assisted selection programmes.

Genotypes derived from genetically similar background cluster together as species exhibit a spatial structure of genetic variation (Ren et al., 2003). The different level of diversity is attributed to the rate of mutation, migration, dispersal mechanisms, biotic and abiotic selection intensities which are determined by location, climate and soil (Kork et al., 1999). The grouping of the genotypes could be attributed to geographical regions or distinct ecotypes that have evolved over the course of rice cultivation. Domestication is a complex evolutionary process in which human use of plant and animal species lead to morphological and physiological changes that distinguish domesticated taxa from their wild ancestors (Hancock, 2005). The cluster obtained was consistent with work on genetic variation in *Prunus Africana* showing that genetic distinctness and differentiation of populations may arise from geographic and ecological isolation (Dawson and Powell, 1999).

Clustering of the interspecifics with *O. sativa* suggested that molecular analysis was unable to identify the parental allele of *O. glaberrima* in these genotypes. Another possibility is that *O. glaberrima* genes were not integrated in the genetic material of the interspecifics because of preferential allele associations (Pharm and Bougerol, 1993) or that the marker was able to identify more of the *O. sativa* alleles in the interspecifics as the markers were specie specific. Clustering of *O. sativa* with *O. glaberrima* have been reported by Semon et al. (2005) that major portions of chromosomes of *O. glaberrima* could not be distinguished from *O. sativa*. The position and organization of two ribosomal RNA gene clusters (45sr DNA and 5sr DNA) were found to be similar in the chromosome of the two species (Ohimido and Fukui,

1995). Today, both *O. glaberrima* and *O. sativa* are commonly grown in mixtures by farmers in upland and rainfed environments. Natural intermediates between the two species have been reported, but the outbreeding rate is estimated to be low (between 2 and 5%) (Jones et al., 1997). Artificial selection tends to enhance population structure and contribute undoubtedly to sub groupings identified in this study. Many accessions of *O. glaberrima* have also been reported to carry genetic evidence of some level of admixture with *O. sativa* which could be accounted for by the introduction of *O. sativa* into West Africa between the 15th and 17th centuries by the Arab traders and Portuguese navigators off the Atlantic Coast (Porteres, 1970).

The genetic profile of *O. glaberrima* is also consistent with the cultural history of rice cultivation pattern in West Africa where *O. glaberrima* is often grown in mixture with *O. sativa*. The low level of diversity within *O. glaberrima* may be attributed uniformly to environmental variables (Buso et al., 1998). As a self pollinating specie that have undergone founder effect, the degree of variation between *O. glaberrima* is expected to exceed the variability observed within species, thus *O. glaberrima* offers an unusual opportunity to detect and characterize the nature of emerging population structure (Jones et al., 1997).

The intra population diversity observed in *O. barthii* were expected since the species is predominatly autogamous (Bezancon, 1977). Accessions of *O. barthii* that out-grouped from other *O. barthii* accessions to cluster with *O. glaberrima* may require further classifications. However, *O. barthii* are progenitors of *O. glaberrima* (Porteres, 1970) and genetic evidence points to a common ancestral gene pool.

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