Genetic Diversity Study of African Rice (*Oryza glaberrima*) and its Wild Relatives using Microsatellites Markers

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Authors' contributions

This work was carried out in collaboration among all authors. Author DM designed the study, wrote the protocol and wrote the first draft. Authors MS and OK performed the analysis of the study. Authors RB and CA revised the protocol and the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The Africa Rice Center Gene Bank hold about 2,500 accessions of *Oryza glaberrima*. To understand well the genetic diversity in *O. glaberrima* and its wild species, the use of molecular tools is prominent. The sample consisted of 217 accessions of *O. glaberrima*, 46 of *O. barthii* and 7 of *O. sativa* (checks) was genotyped with 21 polymorphic microsatellites markers. A total of 245 alleles were detected with average 11.67 alleles per locus. Number of alleles was ranged from 2 (RM124) to 20 (RM536). The polymorphic information content value was 0.49 while the heterozygosity was

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0.091. The result showed that the sample can be clustered into four genotypic groups. Two groups among them were homogeneous. The first one consisted of *O. barthii* accessions with 82 alleles in total with average 3.90 alleles per locus. However, the second one consisted of only *O. glaberrima* accessions with 122 alleles with average 5.80 alleles per locus. *O. glaberrima* accessions were analyzed using model-based population structure. Results revealed two groups among *O. glaberrima* accessions. At the end, the identified core collection has 26 accessions consisted of 16 *O. glaberrima* and 10 *O. barthii* based on 21 microsatellites markers.

Keywords: African rice, alleles, genotypic groups, core collection.

1. INTRODUCTION

African Rice (*Oryza glaberrima*) is an important source for rice genetic improvement [1]. Before the introduction of *O. sativa* varieties by the Portuguese, *O. glaberrima* was domesticated from its wild ancestor (*O. barthii*) during the last 2,000 to 3,000 years in the inland delta of the upper Niger river which is well-known as Mali [2]. This specie was neglected by African farmers because of its low yielding due to high grains shattering and lodging susceptibility [3]. The interest of this specie increased with the finding which showed that *O. glaberrima* can be crossed with *O. sativa* by [4].

Moreover, *O. glaberrima* has many useful traits that can withstand abiotic stress such as, drought tolerance [5], submergence tolerance [6], tolerance to soil acidity, salt and iron toxicity [7,8] and aluminium toxicity [9]. It was also reported that *O. glaberrima* is highly resistant to several diseases such as *Rice yellow mottle virus* [10-12], blast [13], sheath blight, bacterial blight [14], and insect pests, nematodes [15,5], stem borer, hispa and stalked eye fly [16], African gall midge [17,18] and weed competitiveness [19]. Some of the interesting traits of *O. glaberrima* were combined with high-yielding traits of *O. sativa* to develop the "New Rice for Africa" known as NERICA [4]. *O. glaberrima* accessions were considered as a rich genetic source to provide resistant genes to growth constraints. The gene bank of Africa Rice Center holds a collection of about 2,500 samples of African rice which still need to be fully characterized.

Using simple sequence repeats markers developed by [20,21] revealed the population structure of 198 accessions of African Rice with 93 markers. In order to fully exploit the genetic diversity of this specie, the whole collection of 2,500 samples should be characterized and each sample assigned to the right genetic group. After using 74 accessions and 30 microsatellites

markers, three genetic groups of African rice distinguished by [22]. Population structure of *O. glaberrima* was found to be homogeneous after using 20 accessions and 14 unlinked nuclear loci [23]. Likewise, Asian rice domesticated more than 10,000 years ago from *O. rufipogon* was characterized [24]. Therefore, the genetic diversity of *O. sativa* was performed by [25]. Those previous researchers detected five genetic groups corresponding to *indica*, *aus*, *aromatic*, *temperate japonica*, and *tropical japonica* rice. Gene bank of National Institute of Agrobiological Sciences in Japan holds 30,000 accessions of *O. sativa*. Previous study showed 69 varieties as a core collection from the 30000 accessions of *O. sativa* [26]. In order to fully exploit the genetic diversity of this specie, it would be preferable that the collection of 2500 in the Gene bank of the Africa Rice Center could be characterized, and each will be assigned to the right genetic group and identified the core collection. This study aimed to characterize 217 accessions of *O. glaberrima* and its wild ancestor and to establish the mini core collection as well using a set of 21 microsatellites markers.

2. MATERIALS AND METHODS

2.1 Plant Materials

Seeds of all genotypes (*O. glaberrima*, *O. barthii* and *O. sativa*) used in this study were kindly provided by the Gene bank of Africa Rice Center. Two hundred and seventeen accessions of *O. glaberrima* were selected to cover the geographic origins of African rice. Table 1 showed the frequencies based on the geographical distribution of different *O. glaberrima* accessions for this experiment. Additionally, from the Africa Rice Center, 46 accessions of *O. barthii* and 7 of *O. sativa* cultivars (Morobérékan, WAB56-104, Azucena, WITA4, B6144, IR64, and Basmati 370) as checks were used.

Table 1. Distribution of plant material across geographic origins

Origin	Number of Accessions	Frequencies (%)
Burkina Faso	4	1.84
Cameroun	1	0.46
Côte d'Ivoire	13	5.99
Gambia	11	5.07
Ghana	5	2.30
Guinea	8	3.69
Liberia	54	24.88
Mali	14	6.45
Nigeria	94	43.32
Senegal	6	2.76
Sierra Leone	6	2.76
Total	216	99.54
Unknown	1	0.46

2.2 Genotyping

2.2.1 DNA extraction

Before the sowing date, seed dormancy of *O. glaberrima* was broken after removing its coat and cutting the opposite edge of the embryo. Four plantlets per sample were grown per pot (7.5 l each) in the greenhouse in Africa Rice Center (AfricaRice) station based in Cotonou (Bénin). From one month-old plant, young leaves were bulk harvested and frozen at -20 °C until the DNA extraction [27]. Approximately 8 cm of leaf tissue was harvested and folded into 2 mL microfuge tubes above a pool of liquid nitrogen to freeze the tissue. In the AfricaRice biotechnology laboratory, the frozen tissue was crushed while 800 µl of DNA extraction buffer (100mM Tris-HCl, 50mM EDTA, 500mM NaCl, 1.25% (w/v) SDS, 3.8 g per L NaBisulfite) was added. Tubes were vortexed and incubated at 65°C for 20 min. Subsequently, a chloroform extraction was performed with a 24:1 chloroform isoamyl alcohol solution, followed by ethanol precipitation and resuspension in 100 µl of TE buffer. The DNA quality was determined by electrophoresis on 1% agarose gel stained with Gel Red and visualized under an transilluminator (Alpha Imager, AlphaInnotech). The DNA concentration was checked using Nanodrop (Thermo Scientific Nanodrop 8000 spectrophotometer ND-8000 V2.2.1) and the concentration was normalized by dilution to 25 ng/µl.

2.2.2 Capillary electrophoresis

Based on the allele size published on the Gramene website (www.gramene.org/microsat/). 6 multiplex marker sets were composed using

non-overlapping markers labelled with different dyes (Table 2). Markers were developed by [20]. The diluted DNA samples were subjected to PCR amplification (Bio-Rad 580BR 3770) using 21 microsatellites markers (Table 2). Markers were selected basis on the polymorphism they showed for early studies [21]. The sequences of the markers were available on the Gramene website (www.gramene.org/microsat/). The forward primer of each marker was labelled at the 5' end of the oligonucleotide with one of the following fluorescent dyes: 6-FAM (Blue), VIC (Green), NED (Yellow), PET (Red) (Applied Biosystems Inc., USA). PCR amplifications were performed according to the panel using 96-well plates. The total reaction volume of 25 µl consisted of 3 µl DNA, 1.25 µl of each primer (forward and reverse), 12.5 µl of master mix (dNTP, *Taq* DNA polymerase, and Buffer), and 7 µl of pure water. Amplifications were carried out in a thermocycler (Bio-Rad 580BR 3770) using the following program: initial denaturing at 94°C for 5 min followed by 35 cycles of 94°C for 30 s, 55°C annealings for 30 s and 72°C for 30 s and a further primer extension at 72°C for 2 min. Four randomly PCR products were migrated on agarose gel 1% to check the amplification. The master mix of 12 µl consisted of 2 µl diluted PCR product (10%) and 10 µl of formamide and GeneScan-500 LIZ size standard was prepared in optical 96-well MicroAmp plates (Applied Biosystems Inc., USA) for DNA fragments separation during electrophoresis. The master mix was then denatured at 94°C for 5 minutes. Fragment separation was performed using the ABI PRISM 3100 genetic analyzer machine containing POP 4 (performance optimized polymer) matrix and urea following the protocol developed by the AfricaRice Biotechnology unit.

Table 2. Characteristics of selected markers and PCR strategy

Multiplex	Markers	chromosomes	Allele size (pb)	Dye color
1	RM1	1	67 - 143	Green
	RM431	1	233 - 272	Yellow
	RM312	1	86 - 106	Yellow
	RM154	2	148 - 232	Blue
2	RM14643	3	114 - 136	Yellow
	RM338	3	178 - 203	Yellow
	RM514	3	254 - 284	Red
	RM124	4	257 - 290	Yellow
	RM307	4	139 - 193	Green
	RM261	4	119 - 140	Blue
3	RM510	6	129 - 145	Yellow
	RM152	8	133 - 157	Blue
	RM215	9	163 - 173	Green
4	RM552	11	195 - 264	Red
	RM1227	12	141 - 163	Red
	RM19	12	192 - 267	Blue
	RM277	12	104 - 121	Red
	RM219	9	190 - 232	Green
5	RM237	1	105 - 261	Blue
	RM536	11	223 - 247	Green
	RM44	8	111 - 151	Blue

2.3 Molecular data analysis

Molecular data were analyzed using Gene Mapper software version 4.0. (Applied Biosystems Inc., USA) A single or pair of peaks were detected. Fragments sizes were automatically generated with the reference to the internal size standard Gene Scan-500 LIZ from 35 to 500 pb. Eight accessions of *O. glaberrima* were excluded because of a high number of missing data (> 50%). The statistical analysis was run using the 209 accessions and the 3 *O. sativa* checks with the Gen Alex software [28] to eliminate redundant genotypes. Additionally, a genetic dissimilarity matrix was calculated based on the simple matching index using Darwin software [29]. The matrix was used for factorial analysis developed in Darwin. Genetic diversity of the collection, such as the number of alleles (Na), heterozygosity (Ho), Polymorphic Information Content (PIC), and gene diversity were estimated using Power Marker Version 3.25 [30]. The population structure of the samples was inferred using structure software version 2.3.3 [31]. The number of the population tested (K) varied from 1 to 8 within 10 repetitions for each. The procedure was based on the burn-in period of 100000 and 500000 iterations of the Markov-Chain Monte Carlo (MCMC) method [31]. The reel value of K was determined using the method of [32] based on the second-order rate of change

of likelihood of data between consecutive K values. Accessions with a probability of 98% of membership were assigned to the same group.

3. RESULTS

3.1 Genetic Diversity in *O. glaberrima* and *O. Barthii*

Genetic diversity was analyzed with 211 accessions of *O. glaberrima*, 7 varieties of *O. sativa* and 46 accessions of *O. barthii* using 21 microsatellites markers. Similarity analysis of the accessions showed that they differed at least by one allele. As a result, there is no redundant genotype and all genotypes were considered for the analysis.

Allelic diversity analysis revealed a total of 245 alleles with an average of 11.67 alleles per locus. For all markers tested, the number of alleles varied between 2 (RM124) and 20 (RM536). Markers such as RM14643, RM154, RM219, RM1, RM312, RM215, RM19, RM338, and RM536 revealed an above-average number of alleles per locus. The frequency of major alleles varied between 32.06 and 96.96% with an average of 60.23%. In average, 60.23% of the 264 genotypes share a major allele common with each considered locus. The marker RM19 showed the lowest frequency while RM124

showed the highest one. The molecular markers that showed an above-average major allele frequency are: RM307, RM1227, RM154, RM338, RM1, RM14643, and RM124. The H_o value varied between 0 and 50.20% with 9.13% on average. The weakest H_o value was determined by the marker RM124 (0%) while the strongest one was revealed by the marker RM431 (50.2%). The rate of polymorphism varied between 0.0572 (RM124) and 0.7624 (RM312) with 0.4877 on average. Among the 21 markers tested, those which showed a polymorphism rate higher than the average one are: RM277, RM237, RM44, RM431, RM414, RM261, RM219, RM536, RM19, RM312 (Table 3).

3.2 Genotypic Diversity

The result indicated that the samples in this study were divided into four genotypic groups (Fig. 1). Among the groups obtained, two were homogeneous. The genetic diversity (Table 4) for each of these groups was studied.

The first group showed *O. Barthii* accessions with 82 total alleles with average of 3.90 alleles per locus. For the 21 markers tested, the number of alleles per locus varied between 0 and 11. The marker RM536 showed high numbers of alleles

per locus while the markers RM124, RM14643, RM152, and RM1227 showed a number of alleles equal to 0. H_o rate varied between 0 and 61.90% and the marker RM338 indicated the highest amount. An average of 7.4% was obtained for the H_o mean value on the 21 markers tested. Additionally, PIC varied between 0 and 80.63% and RM536 presented the highest value. The lowest rate of polymorphism was obtained using the markers RM124, RM14643, RM152 and RM1227.

The second group pointed out only *O. glaberrima* accessions with 122 alleles and 5.80 alleles per locus as average. The number of alleles varied between 2 and 11. The RM312 marker showed the highest number of alleles while the RM124 and RM552 markers indicated the lowest number of alleles. H_o values ranged between 0 and 0.67 with a mean rate of 0.096. Some molecular markers like RM124, RM552, RM510, and RM215 have shown zero as H_o value. The RM431 marker showed the highest H_o value. Moreover, the polymorphism rate varied between 2.2 and 60% with an average estimated at 0.25 for the markers tested. The marker RM552 was identified as to which with the lowest rate of polymorphism while RM312 had shown the highest polymorphism.

Table 3. Genetic diversity of *O. glaberrima* and *O. Barthii* accessions

Markers	Ma	Na	Gd	Ho	PIC
RM1	0.8372	14	0.2955	0.0426	0.2904
RM154	0.7254	13	0.4593	0.0697	0.4440
RM312	0.3548	14	0.7885	0.2823	0.7624
RM431	0.5080	8	0.6347	0.5020	0.5737
RM124	0.9696	2	0.0590	0.0000	0.0572
RM14643	0.8829	12	0.2157	0.1587	0.2129
RM261	0.4858	8	0.6590	0.0243	0.6047
RM514	0.5240	13	0.6320	0.0320	0.5793
RM307	0.6543	11	0.5088	0.0905	0.4546
RM338	0.7386	19	0.4372	0.2917	0.4182
RM510	0.5837	9	0.5605	0.0195	0.4923
RM152	0.5891	9	0.5447	0.0271	0.4691
RM215	0.5962	14	0.5395	0.0269	0.4646
RM219	0.5382	13	0.6530	0.0305	0.6181
RM19	0.3206	15	0.7360	0.0605	0.6896
RM552	0.5633	11	0.5525	0.0082	0.4696
RM1227	0.6641	10	0.4729	0.0742	0.3969
RM277	0.5884	9	0.5666	0.0281	0.5054
RM44	0.5224	10	0.6105	0.0650	0.5449
RM237	0.5938	11	0.5755	0.0547	0.5241
RM536	0.4087	20	0.7126	0.0278	0.6697
Mean	0.6023	11.667	0.5341	0.0913	0.4877

Ma: Major allele; Na: Number of allele; Gd: Gene diversity;
Ho: heterozygosity; PIC: Polymorphism Information Content

Table 4. Genetic diversity within each group

Marker	Group 1			Group 2			Group 3			Group 4		
	Na	Ho	PIC	Na	Ho	PIC	Na	Ho	PIC	Na	Ho	PIC
RM1	7	0	0.6820	8	0.0674	0.2131	5	0.0303	0.0930	7	0.0625	0.7164
RM154	5	0	0.6721	9	0.0833	0.3623	8	0.0565	0.2949	6	0.1250	0.7114
RM312	4	0	0.5921	11	0.1910	0.6045	12	0.4228	0.7530	4	0.0667	0.5508
RM431	3	0	0.4303	6	0.6705	0.5213	8	0.5040	0.6031	3	0.2	0.5194
RM124	1	0	0	2	0	0.0421	1	0	0	2	0	0.3524
RM14643	1	0	0	7	0.0588	0.0907	9	0.2180	0.2029	6	0.4286	0.5542
RM261	5	0.0526	0.6943	4	0.0128	0.5349	7	0.0226	0.5954	3	0.0588	0.2448
RM514	3	0.1	0.4553	8	0.0500	0.1638	4	0.0150	0.1228	4	0	0.6457
RM307	2	0	0.0866	6	0.2133	0.3226	3	0.0231	0.0374	3	0.1765	0.3749
RM338	9	0.6190	0.7077	7	0.0870	0.2144	9	0.4030	0.3792	6	0.1176	0.4250
RM510	4	0.1429	0.5162	4	0	0.1317	4	0.0149	0.0365	2	0	0.1861
RM152	1	0	0	6	0.0769	0.1812	2	0	0.0150	4	0	0.4791
RM215	3	0	0.1694	6	0	0.1437	2	0.0076	0.0076	8	0.3529	0.7664
RM219	4	0.0476	0.4666	5	0.0761	0.4655	2	0	0.0294	5	0	0.6849
RM19	5	0.1765	0.6833	5	0.0118	0.1209	6	0.0769	0.4284	7	0.0625	0.8033
RM552	3	0.0588	0.1572	2	0	0.0220	2	0.0081	0.0081	6	0	0.7125
RM1227	1	0	0	7	0.1905	0.1971	1	0	0	3	0.1765	0.1993
RM277	2	0.0625	0.0587	3	0.0556	0.3056	1	0	0	6	0.0625	0.6518
RM44	4	0.1875	0.4017	5	0.0930	0.1194	2	0.0391	0.2167	3	0.0000	0.4555
RM237	4	0.0556	0.2797	4	0.0330	0.4005	4	0.0455	0.0587	4	0.2667	0.5464
RM536	11	0	0.8063	7	0.0568	0.1083	8	0.0155	0.4870	5	0	0.5988
Means	3.9048	0.0740	0.3743	5.8095	0.0966	0.2507	5	0.0906	0.2081	5	0.1027	0.5323

Na: Number of allele; Ho: heterozygosity; PIC: Polymorphism Information Content

The group 3 had consisted of both *O. glaberrima* and *O. barthii*. The total number of alleles revealed is equal to 100 with 4.76 alleles per locus on average. The number of alleles varied between 1 and 12 among the investigated samples. Markers RM124, RM1227, and RM277 indicated the lowest number of alleles while RM312 showed the highest one. H_o value fluctuated between 0 and 50% among the samples. Markers RM124, RM1227, RM277; RM152, and RM219 indicated low levels of H_o . The highest level of H_o value was obtained with the marker RM431 with an average of 9.1%. The PIC varied between 0 and 75% with 21% on average. Markers RM124, RM1227 and RM277 showed zero as PIC and the RM312 marker indicated the highest one.

The group 4 includes the three species as *O. glaberrima*, *O. barthii* and *O. sativa*. The total number of alleles revealed was 97 with 4.61 alleles per locus on average. The number of alleles per locus varied between 2 and 8. Markers RM124, and RM510 showed the lowest number of alleles while the marker RM215 indicated the highest one. The average H_o value

determined using the 21 markers tested was estimated at 10.2%. The highest H_o value was recorded on RM14643 (42%) while the lowest one (0%) was determined using markers RM124, RM510, RM44, RM514, RM152, RM219, RM536, and RM552. The rate of polymorphism varied between 19% (RM510) and 80% (RM19) with an average estimated at 53%.

3.3 Allelic Diversity Detected in *O. glaberrima* accessions

Oryza glaberrima evaluated showed 203 as a total number of alleles (N_a). The 21 SSRs used detected 9.66 alleles per locus on average (Table 5). The number of alleles varied from 2 using RM124 marker to 14 using RM312. The frequency of the major allele (M_a) ranged from 0.39 to 0.98 and with a mean value of 0.59. The gene diversity (G_d) ranged from 2.8 to 74.9% with the mean value of 51.3. The heterogeneity (H_o) fluctuated between 0 to 62.4% with 9.9% as an average value. PIC value PIC was estimated at 0.45 and ranged from 0.027 to 0.72.

Fig. 1. Grouping base on 21 markers

3.4 Population Structure of *O. glaberrima*

The *O. glaberrima* accessions used were analyzed by model-based population structure. K value is equal to 2, consequently two populations were revealed in the all accessions tested (Figs. 2 and 3). The limit to assign a sample to a population was fixed at 98%. The first population comprised 92 accessions with 44% of *O. glaberrima*. The genetic diversity indices of the two groups were summarized in Table 6. The two groups showed similar genetic diversity indices means as allele number, major allele frequency gene diversity Ho value and PIC. Nevertheless, some specific differences were found within the two groups based on microsatellites markers. RM19 and RM219 showed a high difference for major allele frequency and gene diversity. Moreover, the allele numbers for RM19, RM219 and RM124 were similar. Additionally, the Ho of markers such as RM312, RM14643 RM307 RM338, and RM1227 allowed the distinction of the two groups. Finally, PIC of RM14643, RM307, RM219, RM237, and RM536 differed for the two groups of *O. glaberrima*.

3.5 Establishment of a Mini Core Collection

The minimum number of accessions that would allow the diversity of the accessions tested to be grouped was established based on the size of alleles. Results revealed a mini core collection consisted of 26 accessions that include 16 *O. glaberrima* and 10 *O. barthii*. Different identified groups during the study are mentioned in the mini core collection. The total number of alleles revealed by the core collection was 123 with an average of 5.86 alleles per locus. The total number of alleles in the core collection was estimated at 50.2% of the initial collection. Some parameters as gene diversity, Ho, and PIC showed similar mean values for both core collection and all samples. Table 7 shows the comparison between the core collection and the global collection.

Table 5. Genetic diversity of 209 accessions of *O. glaberrima* using 21 SSR markers

Markers	Ma	Na	Gd	Ho	PIC
RM1	0.892	9	0.20	0.053	0.196
RM154	0.823	12	0.313	0.066	0.300
RM312	0.425	14	0.749	0.359	0.721
RM431	0.484	8	0.650	0.624	0.590
RM124	0.985	2	0.028	0.000	0.027
RM14643	0.881	12	0.219	0.182	0.215
RM261	0.497	6	0.647	0.025	0.590
RM514	0.515	13	0.595	0.030	0.521
RM307	0.584	11	0.556	0.105	0.485
RM338	0.819	12	0.316	0.237	0.302
RM510	0.573	7	0.520	0.004	0.421
RM152	0.521	8	0.568	0.033	0.481
RM215	0.536	13	0.547	0.019	0.452
RM219	0.521	10	0.641	0.033	0.581
RM19	0.391	10	0.691	0.044	0.632
RM552	0.517	9	0.542	0.005	0.440
RM1227	0.581	10	0.525	0.083	0.432
RM277	0.526	8	0.597	0.029	0.526
RM44	0.468	8	0.616	0.063	0.542
RM237	0.531	10	0.608	0.053	0.545
RM536	0.411	11	0.654	0.034	0.590
Mean	0.594	9.666	0.513	0.099	0.457

Ma: Major allele; Na: Number of allele; Gd: Gene diversity;
Ho: heterozygosity; PIC: Polymorphism Information Content

Fig. 2. Number of population detected

Fig. 3. Ancestries of 212 accessions from 21 SSR

Table 6. Genetic diversity between the two groups

Markers	Group 1					Group 2				
	Ma	Na	Gd	Ho	PIC	Ma	Na	Gd	Ho	PIC
RM1	0.882	8	0.219	0.067	0.213	0.893	7	0.200	0.034	0.196
RM154	0.780	9	0.379	0.083	0.362	0.857	7	0.257	0.045	0.244
RM312	0.534	11	0.645	0.191	0.605	0.332	13	0.799	0.486	0.774
RM431	0.591	6	0.576	0.670	0.521	0.391	8	0.688	0.573	0.626
RM124	0.978	2	0.043	0.000	0.042	0.983	2	0.033	0.000	0.032
RM14643	0.953	7	0.091	0.059	0.091	0.826	11	0.310	0.263	0.300
RM261	0.583	4	0.587	0.013	0.535	0.437	6	0.674	0.025	0.614
RM514	0.913	8	0.166	0.050	0.164	0.882	5	0.216	0.017	0.208
RM307	0.800	6	0.344	0.213	0.323	0.957	5	0.084	0.034	0.083
RM338	0.875	7	0.226	0.087	0.214	0.771	9	0.381	0.350	0.351
RM510	0.929	4	0.135	0.000	0.132	0.983	3	0.033	0.008	0.033
RM152	0.901	6	0.185	0.077	0.181	0.923	3	0.143	0.000	0.135
RM215	0.923	6	0.146	0.000	0.144	0.962	7	0.075	0.026	0.075
RM219	0.527	5	0.558	0.076	0.466	0.924	5	0.145	0.000	0.142
RM19	0.935	5	0.124	0.012	0.121	0.521	5	0.551	0.068	0.454
RM552	0.989	2	0.022	0.000	0.022	0.925	7	0.144	0.009	0.141
RM1227	0.893	7	0.201	0.190	0.197	0.992	3	0.017	0.008	0.016
RM277	0.794	3	0.341	0.056	0.306	0.932	6	0.130	0.009	0.127
RM44	0.936	5	0.122	0.093	0.119	0.809	4	0.322	0.042	0.291
RM237	0.731	4	0.435	0.033	0.400	0.953	6	0.091	0.059	0.090
RM536	0.943	7	0.110	0.057	0.108	0.718	4	0.451	0.017	0.416
Mean	0.828	5.81	0.269	0.097	0.251	0.808	6.0	0.273	0.099	0.255

Ma: Major allele; Na: Number of allele; Gd: Gene diversity; Ho: heterozygosity; PIC: Polymorphism Information Content

Table 7. Comparison between mini core collection (MCC) and global collection (GC)

Markers	Na		Gd		Ho		PIC	
	MCC	GC	MCC	GC	MCC	GC	MCC	GC
RM1	8	14	0.54	0.30	0.04	0.04	0.52	0.29
RM154	7	13	0.61	0.46	0.09	0.07	0.59	0.44
RM312	7	14	0.81	0.79	0.17	0.28	0.78	0.76
RM431	3	8	0.66	0.63	0.33	0.50	0.58	0.57
RM124	2	2	0.27	0.06	0	0	0.23	0.06
RM14643	7	12	0.36	0.22	0.23	0.16	0.35	0.21
RM261	5	8	0.68	0.66	0	0.02	0.63	0.60
RM514	4	13	0.39	0.63	0.04	0.03	0.35	0.58
RM307	8	11	0.68	0.51	0.35	0.09	0.65	0.45
RM338	6	19	0.39	0.44	0.12	0.29	0.36	0.42
RM510	4	9	0.43	0.56	0	0.02	0.40	0.49
RM152	9	9	0.50	0.54	0.08	0.03	0.48	0.47
RM215	8	14	0.67	0.54	0	0.03	0.65	0.46
RM219	6	13	0.77	0.65	0.09	0.03	0.73	0.62
RM19	6	15	0.53	0.74	0	0.06	0.51	0.69
RM552	3	11	0.07	0.55	0.04	0.01	0.07	0.47
RM1227	6	10	0.37	0.47	0.24	0.07	0.36	0.40
RM277	3	9	0.49	0.57	0.13	0.03	0.43	0.51
RM44	6	10	0.45	0.61	0.27	0.07	0.43	0.54
RM237	7	11	0.73	0.58	0	0.05	0.70	0.52
RM536	8	20	0.54	0.71	0.28	0.03	0.53	0.67
Mean	5.86	11.67	0.52	0.53	0.12	0.09	0.49	0.49

Na: Number of allele; Gd: Gene diversity; Ho: heterozygosity; PIC: Polymorphism Information Content

4. DISCUSSION

The genetic diversity of *O. glaberrima* and its wild relatives are important for breeders to improve plants. The molecular characterization study allows to genetically investigate the African rice germplasm. In this study, 21 fluorescent microsatellites markers were tested on *O. glaberrima*. Among the sample evaluated, alleles detecting showed 3 to 14 alleles per locus (9.66 on average) with a total of 203 alleles. The average PIC found was 0.457 while the Ho value was estimated at 0.099. Compared to other studies on *O. glaberrima*, [21] analyzed 198 accessions with 93 microsatellites markers. Their results showed an average of 9.4 alleles per locus with a range from 2 to 27 alleles per locus while the mean PIC value was estimated at 0.34 and the Ho at 0.27. [33] evaluated 26 accessions of *O. glaberrima* using 11 microsatellites markers. Those authors found on average 4 alleles associated with a PIC of 0.45. During their study, [22] discovered that the number of alleles varied from 3 to 19 per locus (8.4 on average), a total number of alleles was estimated at 252 while the mean PIC was 0.52. Those authors used 74 samples of *O. glaberrima* associated with 30 fluorescent microsatellites markers. During their study, [34] found out an average of

4.94 alleles per locus and 0.31 as average PIC values in the *O. glaberrima* collection from Niger.

Results from the model-based population structure analysis showed the presence of two main genotypic groups ($\Delta k = 2$). The structure analysis confirmed the two groups showed by factorial analysis using Darwin. The genetic diversity in each group indicated similar values for average of allele number per locus, Ho and PIC. In comparison to previous studies, [21] found out $\Delta k = 5$ which indicated five groups within the 198 *O. glaberrima* accessions. The difference between both studies can be explained by the highest density of markers for this purpose (93 vs 21). Previous studies revealed that it is a correlation between the number of genomic structures and density of markers. [35] showed that response obtained when increasing the marker density is largest when using a diverse training set to predict between poorly related material. Among the five genetically distinct groups, two admixtures groups were also identified sharing ancestry with *O. sativa* species. [22] also discovered three populations using 74 accessions and 30 SSR markers. The population structure mentioned by [36] was homogeneous with no obvious genetic subdivision. The present work indicated low

diversity in *O. glaberrima* specie and confirmed previous findings. According to [36], the low genetic diversity of *O. glaberrima* would have been a genetic bottleneck during its domestication from a small initial population of *O. barthii*. The low diversity in *O. glaberrima* can also be explained by the low genetic diversity in *O. barthii*, the immediate ancestor of African rice, because *O. barthii* possessed the lowest nucleotide diversity for a wild plant species as reported by [37] and [38]. The low nucleotide diversity in *O. glaberrima* can therefore be explained by the severe genetic bottleneck it experienced during domestication [36]. Otherwise, self-pollination was found to be another factor leading to low diversity of *O. barthii* (wild of *O. glaberrima*) because it reduces effective population size and effective recombination rates and thus decreases diversity [39].

The mini core collection obtained from the global set gave 26 accessions including 16 *O. glaberrima* and 10 *O. barthii* based on 21 microsatellites markers. The mini core collection identified can represent the whole accessions tested as show the diversity index; Gd (0.52 vs 0.53), Ho (0.12 vs 0.09) and PIC (0.49 vs 0.49). The core collection is a limited set of accessions to represent the genetic spectrum of the whole collection [40]. In the end, 69 accessions were selected for the collection based on RFLP data from 332 accessions of *O. sativa* [26]. [41] were the first to highlight the need for a reference collection and suggest the idea of a "Core collection". According to these authors, setting up a core collection consists of obtaining a sub-sample of the basic collection presenting a maximum of diversity. With 10% of a collection, it is possible to conserve 75% of allelic diversity [42]. The long-term maintenance of the basic collections and their circulation between the continents justifies the creation of a core collection. The size of a core collection has long been a subject of debate [41]. However, in this study number of an allele in core collection is about 50% of the number of an allele of the global collection tested. The number of markers used should be increased to access more relevant information on the genetic diversity of *O. glaberrima* and its wild accessions.

5. CONCLUSION

Nowadays, African rice (*Oryza glaberrima*) becomes meaningful in breeding programs, but its genetic diversity is not well documented. This

study focused on the genetic diversity assessment of 217 accessions from *O. glaberrima*; and 46 from *O. barthii* one using 21 microsatellites markers. Cluster results revealed 4 genotypic groups, while two were homogeneous based on allele frequencies. At the end, a mini core collection consisted of 26 accessions identified as representative of all tested accessions was established. This finding is a great contribution to rice improvement to control abiotic and biotic stresses which affect its production.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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