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Genetic diversity assessment of lowland and upland rice varieties of Mali using microsatellite markers

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Rice (*Oryza sativa*) is a crucial staple food globally, and understanding the genetic diversity of both lowland and upland local rice varieties is essential for their effective management, conservation, and efficient utilization in varietal improvement programs. In the present study, a total of 58 rice varieties, comprising 9 uplands and 49 lowlands, were sourced from the Lowland Rice Breeding Program of the Regional Center for Agronomic Research of Sikasso at the Institute of Rural Economy. These genotypes were characterized using polymerase chain reaction-simple sequence repeat (PCR-SSR) with 101 microsatellite markers. A total of 428 alleles were recorded and the number of alleles per locus varied from 2 (RM5, RM212, RM243, RM302, RM1387, RM240, RM250, RM517, RM249, RM3330, RM3826, RM339, RM205, RM257, RM5786 and RM235) to 10 (RM009 and RM481) with an average of 4.24 alleles per locus. The Polymorphism Information Content (PIC) value was ranged from 0.120 (RM145) to 0.832 (RM5780) with an average of 0.459. Forty percent (40%) of the markers were highly informative, 44% reasonably informative and 16% slightly informative. The value of genetic diversity revealed by the markers was ranked from 0.128 to 0.848 with an average of 0.497 for the same markers as the PIC. Similarity analysis based on genetic distance allowed classifying the 58 genotypes into three groups (I, II and III). Groups I and III were formed only by traditional lowland varieties collected from rice farmers. Groups I and III exclusively comprised traditional lowland varieties obtained from rice farmers, while Group II included upland rainfed interspecific varieties like *O. sativa*, *Oryza glaberrima*, *Oryza longistaminata*, alongside some traditional lowland varieties such as Diema2, Sikasso56, Sikasso-1-14, Diama20, Diola, and Yorosso200. These findings offer valuable insights for breeders in managing rice genebanks, identifying lowland varieties with unknown genetic origins, and selecting lines with desirable agronomic traits for rice improvement programs.

Key words: Genetic diversity, rice varieties, lowland, upland, polymerase chain reaction-simple sequence repeat (PCR-SSR).

INTRODUCTION

Rice is a vital staple food in West Africa, constituting 37% of cereal consumption. Mali ranks as the second-largest rice producer after Nigeria (Tondel et al., 2020). Lowland, upland, and free submersion rice cultivation hold

immense potential across all regions of Mali, covering an estimated area of about 980,000 ha (FAO, 2010). Projected total paddy production from lowlands rice is expected to range from 570,000 to 1.2 million tons between 2020 and 2030 (Ouédraogo et al., 2021). Varieties cultivated in lowlands often belong to *Oryza sativa*, domesticated for an extended period and currently categorized as local varieties. In free submersion rice cultivation, mainly African rice (*Oryza glaberrima*) varieties are grown. Traditional varieties, often non-dormant and photosensitive, allow for dual harvesting from different water fringe areas. Most rice varieties, predominantly African rice (*O. glaberrima*), are cultivated under free submersion conditions. Recently, researchers have developed several rice varieties adapted to these production systems by crossing the two cultivated species, *O. sativa* and *O. glaberrima (*Ministère de l'agriculture, 2020*)*.

The most cultivated are the NERICA (New Rice for Africa), WITA, and ARICA lines (Bureau de Coordination Technique du TAAT, 2021). Farmers have accessed to these varieties introduced from varietal demonstration tests conducted by researchers. In some lowlands, there are seed multiplier farmers whose production is purchased, stored, and then sold to other farmers (ODI, 2001) often attributing local name to the new variety. This uncontrolled seed production can result in the introduction of exogenous genes. This is how several local varieties of unknown origin, called under the name of the locality of provenance were collected and stored by lowland rice program of Sikasso. The genetic characterization of these genetic resources in general could increase their usefulness in the breeding program (Bajracharya et al., 2006), with a view to better conservation and sparing use of plant genetic resources and a perspective of varietal innovation (Moukoumbi, 2012; Mohammadi-Nejad et al., 2008; Teklu et al., 2006). Polymerase chain reaction (PCR) based on simple sequence repeat (SSR) markers is an important tool for the assessment of genetic variation in plant genetic resources (Mishra et al., 2022). Due to the advantages like reproducibility, codominance, high polymorphism, SSR markers are more used than any other type of DNA molecular markers (Xue et al., 2012). Several studies of genetic diversity related to rice collection using SSR markers have already been carried out around the world. Recently, Yang et al. (2021), Sujan et al. (2021) and Hoque et al. (2021) assessed the genetic diversity *Oryza sativa* collection in Island, India and Bangladesh, respectively. In Mali, Dao et al. (2018) evaluated the genetic variability of 54 irrigated rice varieties. But very few genetic characterization studies have been focused exclusively on rice used in lowland and upland rice cultivation. Our objective was to study the genetic diversity of rice varieties provided by the Lowland Rice Program of the Regional Center for Agronomic Research using microsatellite markers.

MATERIALS AND METHODS

Plant

Fifty-eight rice varieties, comprising nine upland and forty-nine lowland varieties, obtained from the Lowland Rice Program of the Regional Center for Agronomic Research in Sikasso, were subjected to genetic diversity analysis (Table 1). These varieties encompass three distinct species: *O. sativa, O. glaberrima,* and *Oryza longistaminata*, as well as interspecific hybrids resulting from crosses between *O. sativa* and *O. glaberrima*. Among them, thirty varieties were sourced from local growers across different locations, their genetic origins unknown, and are classified as traditional genotypes bearing the names of their collection sites. The remaining varieties are officially recognized and listed in the Official Catalog of Plant Species and Varieties of Mali (2020). Seeds of each variety were stored at the Research Laboratory in Microbiology and Microbial Biotechnology (LaboREM-Biotech) at the Faculty of Sciences and Techniques of the University of Sciences, Techniques and Technologies of Bamako for subsequent analysis.

DNA extraction

The DNA extraction from the leaves, collected at the juvenile stage of 15-day-old plants, was performed using the cetyltrimethylammonium bromide (CTAB) protocol following the method described by Dao et al. (2018). The obtained DNA pellets were subjected to two washes with 70% ethanol through centrifugation at 10,000 rpm for 30 s each, followed by drying for two hours at room temperature. Subsequently, 100 µl of 0.5 M Tris EDTA (TE) buffer was added to dissolve the DNA. To remove RNA contamination, 1 µl of RNase Promega was added to the diluted DNA and the mixture was incubated at 37°C for 15 min. DNA quantification and quality assessment were conducted using spectrophotometry at 260 nm (for nucleic acids) and 280 nm (for proteins) to determine the DO260/DO280 ratio, as described by Raoudha et al. (2012). This ratio serves as an indicator of DNA purity and potential protein contamination, with reference to the DO260/DO280 ratio interval reported by Ayed et al. (2019). The resulting DNA samples were diluted to a final concentration of 20 ng/µl and stored at -20°C for subsequent PCR-SSR analysis.

DNA amplification

The extracted DNA samples were subjected to Polymerase Chain Reaction (PCR) amplification using a panel of 101 SSR markers selected based on their polymorphism and chromosomal distribution (Table 2). Additional details regarding the SSR markers, including their sequences, chromosome numbers, repeat patterns, and hybridization temperatures, can be accessed through the Gramene website (http://www.gramene.org), as well as the protocol established by McCouch et al. (2002). The PCR reaction mixture and cycling program followed the method outlined by Dao et al. (2018).

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Table 1. List of 58 rice genotypes used in the study.

Code	Name	Varietal type	Rice cultivation type	Code	Name	Varietal type	Rice cultivation type
1R	NERICA 4	*O. sativa* X *O. glaberrima*	Upland	30R	Dièma 2	-	Lowland
2R	Kolondièba 10	-	Lowland	31R	Sikasso 56	-	Lowland
3R	Kadiolo 32	-	Lowland	32R	DKA 1	*O. sativa* X *O. glaberrima*	Irrigated/Lowland
4R	Dioila 5	-	Lowland	33R	DKA 4	*O. sativa* X *O. glaberrima*	Lowland
5R	Yorosso 21	-	Lowland	34R	DKA 21	*O. sativa* X *O. glaberrima*	Lowland
6R	Sikasso 1	-	Lowland	35R	DKA 23	*O. sativa* X *O. glaberrima*	Lowland
7R	Bougouni 24	-	Lowland	36R	DKA 30	-	Lowland
8R	Dièma 14	-	Lowland	37R	DKA 31	-	Lowland
9R	Niena 1-2	-	Lowland	38R	DKA M7	*O. longistaminata*	Lowland
10R	Kignan 4	-	Lowland	39R	DKA M11	*O. longistaminata*	Lowland
11R	OHVN 4	-	Lowland	40R	DKA M13	*O. longistaminata*	Lowland
12R	OHVN 17	-	Lowland	41R	BW 348-1	*O. sativa*	Lowland
13R	Yanfolila 4	-	Lowland	42R	KAODAWK Mali105	*O. sativa*	Lowland
14R	Yanfolila 20	-	Lowland	43R	Mut 93-2-2-1-1-4	*O. glaberrima*	Lowland
15R	Danderesso 10-2	-	Lowland	44R	ARICA 3	*O. sativa* X *O. glaberrima*	Lowland
16R	Dissigre	-	Lowland	45R	Sik 305-A-150	*O. sativa*	Lowland
17R	Garalo 2	-	Lowland	46R	Sik 353-A-10	*O. sativa*	Lowland
18R	Kangaba 11-2	-	Lowland	47R	NERICA 8	*O. sativa* X *O. glaberrima*	Upland
19R	Crete	-	Lowland	48R	NERICA 9	*O. sativa* X *O. glaberrima*	Upland
20R	Finkolo 5	-	Lowland	49R	NERICA 12	*O. sativa* X *O. glaberrima*	Upland
21R	Zangasso	-	Lowland	50R	DKA P17	*O. sativa* X *O. glaberrima*	Upland
22R	Kayes 31	-	Lowland	51R	DKA P27	*O. sativa* X *O. glaberrima*	Upland
23R	Kadiolo 14	-	Lowland	52R	DKA P28	*O. sativa* X *O. glaberrima*	Upland
24R	Dioila 3	-	Lowland	53R	DKA P29	*O. sativa* X *O. glaberrima*	Upland
25R	Kolondièba 6	-	Lowland	54R	DKA P30	*O. sativa* X *O. glaberrima*	Upland
26R	Yorosso 15-2	-	Lowland	55R	Diola	-	Lowland
27R	Niena 6-3	-	Lowland	56R	Yorosso 200	-	Lowland
28R	Bougouni 32	-	Lowland	57R	Dièma 20	-	Lowland
29R	Koumantou 3	-	Lowland	58R	Sikasso-1-14	-	Lowland

Electrophoresis and visualization of PCR products

Approximately 10 µl of the PCR products were loaded onto a CONDA MS-4 3% (w/v) agarose gel, which is specifically designed for the separation of DNA fragments smaller than 500 bp. Electrophoresis was conducted for 2 h and 30 min at 80 V. The gel was prepared using a solution of 0.5X TBE (Tris, Boric acid, EDTA) buffer supplemented with 30 µl of 10% ethidium bromide (mg/ml). Subsequently, the gel was visualized and photographed under ultraviolet light using the E-BOX VX2 version 15.06 gel documentation systems.

Data analysis

DNA fragments appear as bands (fluorescence) on the agarose gel. The size of these bands was determined in base pairs using E-Capt version 15.06 software, by comparison with the standard marker Promega 50bp DNA

Table 2. Chromosome number, names of the 101 SSR primer pairs.

No.	No. Chr	SSR marker	No.	No. Chr	SSR marker	No.	No. Chr	SSR marker
1	1	RM1	35	4	RM1359	69	8	RM339
2	1	RM5	36	4	RM5687	70	8	RM447
3	1	RM009	37	4	RM5714	71	8	RM515
4	1	RM212	38	4	RM6089	72	8	RM1376
5	1	RM243	39	5	RM249	73	8	RM5545
6	1	RM246	40	5	RM289	74	9	RM201
7	1	RM263	41	5	RM334	75	9	RM205
8	1	RM302	42	5	RM413	76	9	RM242
9	1	RM475	43	5	RM430	77	9	RM257
10	1	RM580	44	5	RM1024	78	9	RM1026
11	1	RM1387	45	5	RM1248	79	9	RM3744
12	1	RM3252	46	6	RM190	80	9	RM3912
13	2	RM145	47	6	RM225	81	9	RM5786
14	2	RM211	48	6	RM253	82	9	RM6021
15	2	RM240	49	6	RM276	83	10	RM222
16	2	RM250	50	6	RM508	84	10	RM258
17	2	RM324	51	6	RM527	85	10	RM333
18	2	RM341	52	6	RM541	86	10	RM496
19	2	RM1063	53	6	RM584	87	10	RM590
20	2	RM1367	54	6	RM3330	88	10	RM6824
21	2	RM5780	55	7	RM010	89	11	RM21
22	3	RM016	56	7	RM11	90	11	RM167
23	3	RM135	57	7	RM18	91	11	RM202
24	3	RM218	58	7	RM125	92	11	RM206
25	3	RM251	59	7	RM214	93	11	RM224
26	3	RM282	60	7	RM481	94	11	RM244
27	3	RM517	61	7	RM3404	95	11	RM286
28	3	RM5626	62	7	RM3826	96	11	RM287
29	4	RM252	63	7	RM6697	97	12	RM17
30	4	RM255	64	7	RM6767	98	12	RM19
31	4	RM273	65	8	RM025	99	12	RM101
32	4	RM470	66	8	RM072	100	12	RM235
33	4	RM518	67	8	RM152	101	12	RM247
34	4	RM551	68	8	RM223			

Chrs: Chromosome, SSR: Simple Sequence Repeat.

Step Ladder.

The diversity of each variety was assessed based on allele frequency, number of alleles, genetic diversity, and PIC (Polymorphism Information Content) (Shakil et al., 2015). Each DNA band represented a homozygous allele, and each marker represented a locus. A matrix of 1s and 0s, indicating the presence and absence of an allele respectively, was created and analyzed using Power Marker version 3.25 software (Shakil et al., 2015; Lin et al., 2012) to calculate allele frequency, number of alleles, genetic diversity, and Polymorphism Information Content (PIC).

The PIC value for each marker was used to assess polymorphic information. An average PIC value across all genotypes reflects the genetic diversity within the group. If the PIC value is > 0.5, the marker is considered highly informative; if it falls between 0.25 and 0.5 (0.5 > PIC > 0.25), it is reasonably informative; and if the PIC value is < 0.25, the marker is considered slightly informative (Botstein et al., 1980). Genetic distance was calculated using the method of Nei (1983), and a phylogenetic tree based on genetic distance was constructed using the Neighbor-Joining method with Power Marker version 3.25 software. The tree was displayed and edited using MEGA version 7 software (Dao et al., 2018). The genetic distance matrix of Nei (1983) was also used to generate a principal component plot with GenAlEx 6.51b2 software.

RESULTS

Polymorphism of SSR markers

Genetic analysis of 58 lowland and upland rice varieties using 101 SSRs revealed a total of 428 alleles. The

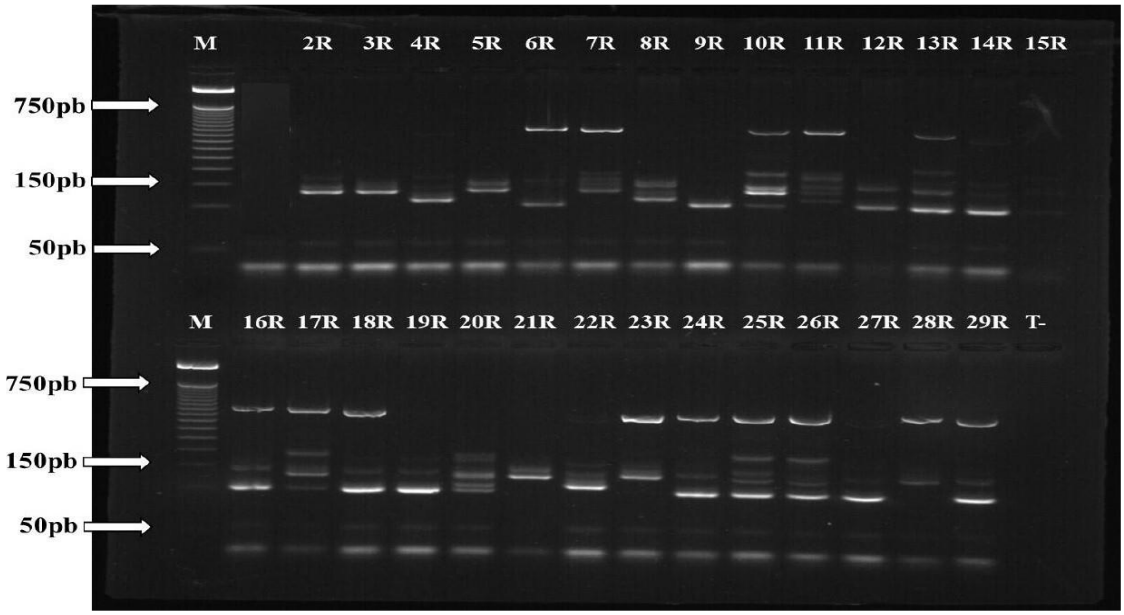

Figure 1. DNA profile of 28 rice genotypes with RM5780 marker on Agarose MS-4 after 2 h 30 min of migration. M : DNA Standard Marker, 2R : Kolondièba 10, 3R : Kadiolo 32, 4R : Dioila , 5R : Yorosso 21, 6R : Sikasso 1, 7R : Bougouni 24, 8R : Dièma 14, 9R : Niena 1-2, 10R : Kignan 4, 11R : OHVN 4, 12R : OHVN 17, 13R : Yanfolila 4, 14R : Yanfolila 20, 15R : Danderesso 10-2, 16R : Dissigre, 17R : Garalo 2, 18R : Kangaba 11-2, 19R : Crete, 20R : Finkolo 5, 21R : Zangasso, 22R : Kayes 31, 23R : Kadiolo 14, 24R : Dioila 3, 25R : Kolondièba 6, 26R : Yorosso 15-2, 27R : Niena 6-3, 28R : Bougouni 32, 29R : Koumantou 3, T- : negative control.

number of alleles ranged from 2 (RM5, RM212, RM243, RM302, RM1387, RM240, RM250, RM517, RM249, RM3330, RM3826, RM339, RM205, RM257, RM5786, and RM235) to 10 (RM009 and RM481), with an average of 4.24 per locus (Table 3).

All SSR markers exhibited polymorphism, with the highest frequency value (0.93) observed for RM580, RM145, RM517, and RM339 markers. The PIC value varied from 0.120 (RM145) to 0.832 (RM5780) (Figure 1), with an average of 0.459. Among the markers, 40% were highly informative, 44% were reasonably informative, and 16% were slightly informative. The genetic diversity value revealed by the markers ranged from 0.128 (RM145) to 0.848 (RM5780), with an average of 0.497 for the same markers as the PIC.

The highest average number of alleles per locus (5.5) was observed on chromosome 7, while chromosome 2 exhibited the highest average PIC value (0.528) (Table 4).

Genetic relationship between rice varieties

The dendrogram, based on genetic similarities among the 58 rice genotypes, classified the varieties into three groups (I, II, and III) (Figure 2). Groups I and III consisted solely of traditional lowland varieties collected from growers. Group II comprised all upland varieties (NERICA 4, NERICA 8, NERICA 9, NERICA 12, DKA P17, DKA P27, DKA P28, DKA P29, DKA P30), some lowland varieties (DKA1, DKA4, DKA21, DKA23, DKA30, DKA31, and ARICA 3), interspecific varieties, *O. sativa* varieties (KAODAWK Mali 105, BW348-1, Sik353-A-10, and Sik305-A150), *O. glaberrima* varieties (Mut93-2-2-1-1-4), *O. longistaminata* varieties (DKAM7, DKAM13, and DKAM11), and some traditional varieties of unknown genetic origin (Kaye 31, Crete, Sikasso-1-14, Diema 20, Diola, Yorosso 200, Sikasso 56, and Diema 2).

However, the interspecific upland varieties were genetically very close and formed a separate cluster closely associated with four varieties of unknown genetic origin: Sikasso-1-14, Diema 20, Diola, and Yorosso 200. These findings suggest that these varieties could be used interspecifically by growers. Group II comprised 55% of the genotypes studied, followed by group III, which grouped together 35%.

Principal component analysis

Principal Component Analysis (PCA) was conducted using the genetic distance matrix of Nei (1983) based on 101 SSR markers. The analysis revealed that the first two principal components accounted for 12.58 and 7.64% of the genetic variation, respectively, with a total variation of 20.22%. All varieties were classified into three groups,

Table 3. Number of alleles, allele frequency, genetic diversity and PIC of 58 rice varieties.

Marker	No. Chrs	Number of alleles	Major Allele frequency	Allele No.	Gene diversity	PIC
RM1	1	5	0.28	8	0.795	0.767
RM5	1	2	0.84	2	0.262	0.228
RM009	1	10	0.31	8	0.783	0.753
RM212	1	2	0.74	3	0.417	0.379
RM243	1	2	0.86	3	0.247	0.233
RM246	1	4	0.38	7	0.706	0.658
RM263	1	3	0.41	6	0.671	0.613
RM302	1	2	0.69	3	0.476	0.428
RM475	1	6	0.71	6	0.467	0.433
RM580	1	5	0.93	5	0.132	0.130
RM1387	1	2	0.88	2	0.212	0.190
RM3252	1	4	0.45	6	0.697	0.650
RM145	2	8	0.93	2	0.128	0.120
RM211	2	4	0.62	8	0.584	0.559
RM240	2	2	0.72	3	0.432	0.386
RM250	2	2	0.78	3	0.373	0.342
RM324	2	5	0.45	7	0.726	0.693
RM341	2	8	0.38	12	0.760	0.729
RM1063	2	3	0.79	3	0.340	0.301
RM1367	2	8	0.33	9	0.812	0.789
RM5780	2	7	0.26	15	0.848	0.832
RM016	3	7	0.52	9	0.648	0.602
RM135	3	7	0.76	2	0.366	0.299
RM218	3	3	0.53	3	0.605	0.536
RM251	3	6	0.28	11	0.832	0.811
RM282	3	3	0.62	4	0.495	0.403
RM517	3	2	0.93	3	0.130	0.125
RM5626	3	4	0.74	5	0.424	0.396
RM252	4	5	0.33	9	0.820	0.801
RM255	4	3	0.76	3	0.393	0.356
RM273	4	4	0.47	6	0.675	0.623
RM470	4	6	0.79	5	0.353	0.331
RM518	4	4	0.45	5	0.607	0.528
RM551	4	3	0.64	5	0.505	0.434
RM1359	4	3	0.48	5	0.638	0.575
RM5687	4	6	0.41	6	0.690	0.638
RM5714	4	4	0.69	3	0.447	0.373
RM6089	4	4	0.67	6	0.518	0.490
RM249	5	2	0.81	3	0.325	0.301
RM289	5	2	0.57	2	0.490	0.370
RM334	5	4	0.91	4	0.162	0.158
RM413	5	4	0.29	8	0.793	0.764
RM430	5	3	0.86	3	0.247	0.232
RM1024	5	5	0.76	4	0.402	0.377
RM1248	5	3	0.76	4	0.398	0.367
RM190	6	3	0.69	4	0.474	0.425
RM225	6	5	0.41	7	0.743	0.708
RM253	6	6	0.67	3	0.476	0.411
RM276	6	3	0.48	7	0.698	0.663
RM508	6	4	0.79	5	0.355	0.335
RM527	6	3	0.62	4	0.506	0.422

Table 3. Cond.

RM541	6	4	0.48	4	0.599	0.517
RM584	6	4	0.59	6	0.565	0.501
RM3330	6	2	0.78	2	0.348	0.287
RM010	7	6	0.74	5	0.429	0.406
RM11	7	4	0.62	6	0.576	0.545
RM18	7	9	0.34	12	0.803	0.781
RM125	7	6	0.45	8	0.736	0.708
RM214	7	5	0.86	7	0.253	0.246
RM481	7	10	0.40	7	0.692	0.637
RM3404	7	4	0.76	7	0.410	0.392
RM3826	7	2	0.86	2	0.238	0.210
RM6697	7	6	0.74	8	0.435	0.417
RM6767	7	3	0.84	5	0.279	0.268
RM025	8	3	0.53	4	0.634	0.585
RM072	8	4	0.33	8	0.802	0.777
RM152	8	9	0.38	16	0.788	0.766
RM223	8	5	0.41	8	0.757	0.730
RM339	8	2	0.93	4	0.131	0.128
RM447	8	3	0.60	4	0.557	0.498
RM515	8	3	0.62	3	0.483	0.382
RM1376	8	3	0.81	3	0.318	0.285
RM5545	8	4	0.76	4	0.404	0.381
RM201	9	4	0.90	4	0.191	0.182
RM205	9	2	0.57	3	0.540	0.451
RM242	9	5	0.50	3	0.600	0.520
RM257	9	2	0.86	3	0.247	0.233
RM1026	9	3	0.88	2	0.212	0.190
RM3744	9	5	0.74	2	0.383	0.310
RM3912	9	4	0.72	3	0.416	0.354
RM5786	9	2	0.72	5	0.446	0.414
RM6021	9	7	0.36	7	0.759	0.722
RM222	10	5	0.41	6	0.702	0.650
RM258	10	9	0.90	3	0.190	0.181
RM333	10	4	0.78	5	0.376	0.349
RM496	10	5	0.57	7	0.616	0.575
RM590	10	3	0.79	5	0.355	0.335
RM6824	10	4	0.72	6	0.454	0.432
RM21	11	3	0.41	5	0.702	0.652
RM167	11	3	0.78	3	0.355	0.303
RM202	11	3	0.66	4	0.505	0.447
RM206	11	8	0.55	6	0.617	0.566
RM224	11	4	0.74	7	0.433	0.413
RM244	11	4	0.74	5	0.421	0.388
RM286	11	4	0.45	6	0.670	0.613
RM287	11	5	0.55	8	0.656	0.630
RM17	12	3	0.45	3	0.647	0.574
RM19	12	3	0.57	4	0.587	0.527
RM101	12	3	0.90	4	0.192	0.185
RM235	12	2	0.78	4	0.376	0.350
RM247	12	3	0.84	4	0.274	0.257
Mean	-	-	-	-	0.497	0.459
Total	-	428	-	-	-	-

PIC: Polymorphism information content.

Table 4. Number of SSR marker, average number of alleles, average genetic diversity and average PIC per chromosome.

No. Chrs	Number of SSR marker	Average number of alleles	Average genetic diversity	Average PIC
Chrs 1	12	3.91	0.489	0.455
Chrs 2	9	5.22	0.556	0.528
Chrs 3	7	4.57	0.500	0.453
Chrs 4	10	4.2	0.565	0.515
Chrs 5	7	3.29	0.403	0.367
Chrs 6	9	3.77	0.529	0.474
Chrs 7	10	5.5	0.485	0.461
Chrs 8	9	4	0.542	0.504
Chrs 9	9	3.77	0.422	0.375
Chrs 10	6	5	0.449	0.420
Chrs 11	8	4.25	0.545	0.502
Chrs 12	5	2.8	0.415	0.379
Total	101	-	-	-
Moyenne	8.42	-	-	-

Chrs: Chromosome, SSR: Simple Sequence Repeat; PIC: Polymorphism Information Content.

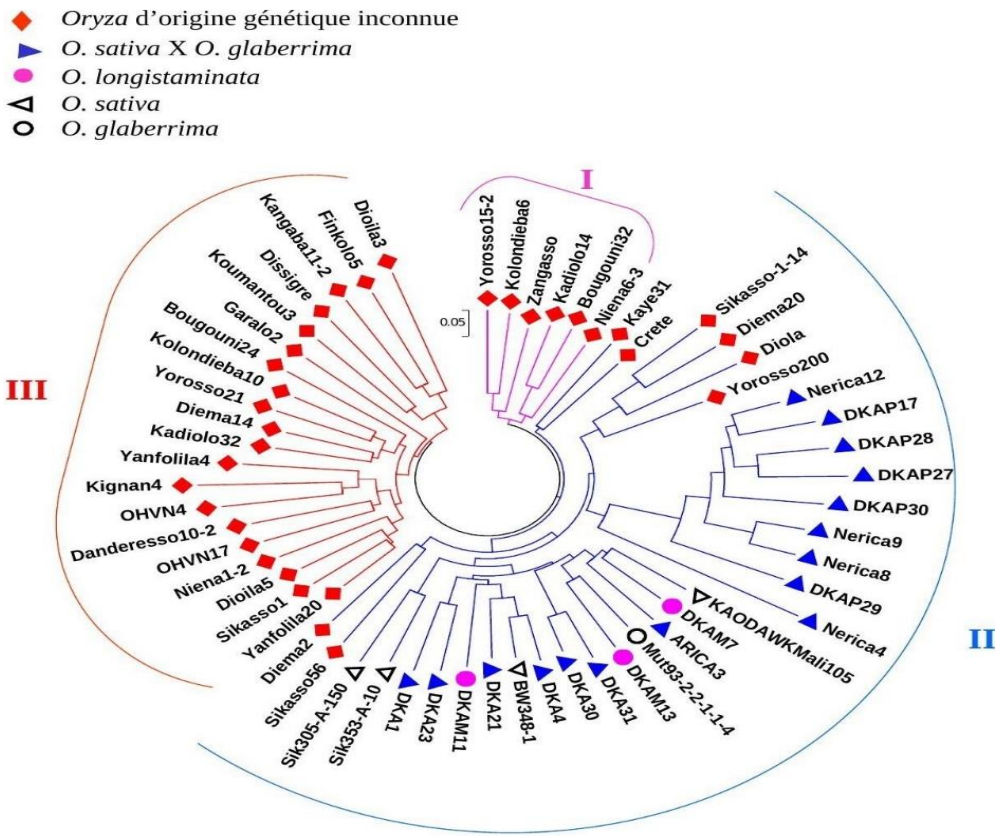

Figure 2. Neighbor joining tree based on genetic distances Nei (1983) between 58 rice genotypes using 101 SSR markers.

with a few isolated genotypes (Figure 3). Group I consisted of interspecific varieties adapted to upland rice cultivation. Group II comprised a mixture of varieties of *O. sativa, O. glaberrima, O. longistaminata*, and interspecific

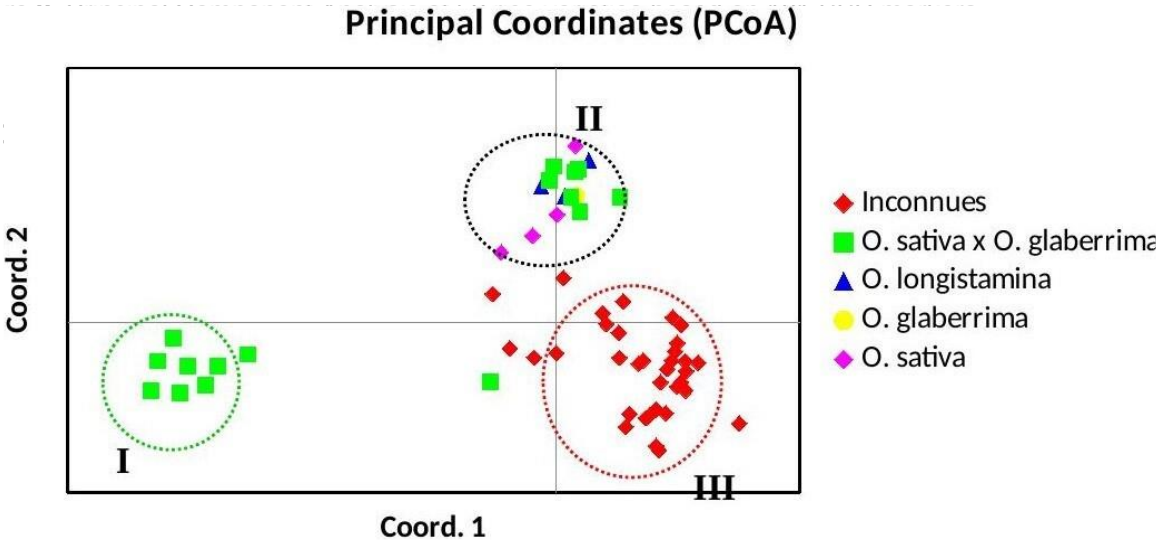

Figure 3. Principal Component Analysis of 58 rice varieties based on 101 SSR markers.

hybrids. Group III exclusively included varieties of unknown genetic origin.

DISCUSSION

The molecular analysis of rice collection revealed high genetic diversity within and between rice species. This diversity could be linked to the origin of the varieties, a mixture of three species of genus Oryza. Several varieties are interspecific such as NERICA, ARICA, DKA P used in upland rice cultivation and some DKA (Ministère de Agriculture, 2020). In addition, the seeds of these traditional varieties collected in the different localities are often produced under inappropriate conditions by seed multiplier farmers (ODI, 2001) which may lead to the introduction of exogenous genes. Although the genetic origin (parents) of certain local varieties is not known, the high genetic diversity observed could be due to the growing conditions and particularly to the conditions of seed production. The total number of alleles obtained is superior to that of Ogunbayo et al. (2021) who characterized 48 lowland rice genotypes including 37 interspecific (*O. glaberrima* × *O. sativa* ssp. *indica*) and 11 intraspecific (*O. sativa* ssp. indica × *O. sativa* ssp. *indica*) with 50 SSR makers. These authors identified 10 polymorphic SSRs (loci) that produced a total of 49 alleles with an average of 4.9 per marker. The difference between the two studies in terms of polymorphic loci observed could be related to the use of varieties belonging to the species *O. longistaminata* and to genotypes of unknown genetic origin. Pathaichindachote et al. (2019) obtained 110 alleles with an average of 8.5 per marker. This average is superior than that of current study which difference could be related to the collection size with 83% upland rice and 17% lowland rice with all belonged to the species *O. sativa*. Genotypes composed of intra- and interspecific varieties produced 0.61 as PIC average generated from 14 SSRs (Suvi et al., 2020) which is beyond to the PIC of actual study despite the low number of SSR markers used. Similarly, Chuchert et al. (2022) recorded a PIC varying from 0.25 (RM215) to 0.76 (RM413) with an average of 0.63 by evaluating the diversity level of 44 upland varieties of unknown genetic origin with 10 SSR. The loci RM252 and RM253 loci, located on chromosomes 5 and 6 respectively, were highly informative and exhibited the same number of alleles in 98 upland varieties collected from northern Thailand (Nilthong et al., 2020). In addition, the loci RM289 and RM19, polymorphic with respective PIC of 0.370 and 0.527, were revealed monomorphic by Yang et al. (2021) following a study of 57 upland accessions.

The loci located on chromosome 7 presented the greatest average (5.5) of allele per locus while those of chromosome 2 revealed the highest value of the average PIC (0.528). In a study carried out by Tamuly et al. (2022) on 50 upland rice accessions, all the chromosomes presented an average number of alleles different from that of current study with the exception of chromosome 12, which recorded the same average (2.8) with also 5 SSR markers used. In addition, the loci RM212, RM243, RM517, RM257 and RM19 located respectively on chromosome 1, 1, 3, 9 and 12 presented the same number of alleles as the case of our study. Puspito et al. (2022) obtained an average PIC of 0.53 at chromosome 2 loci following an analysis of the genetic diversity of 50 local rice varieties of *O. sativa* species. This average is very close to that recorded in this study despite the difference between the structures of the populations

studied. These same authors had the highest average of PIC (0.665) on chromosome 12. The varieties of each group have a strong similarity between them. The varieties of group I and III were all of unknown genetic origin and a few were found in group II with very diverse level. This genetic differentiation could be due to the acquisition of new alleles, linked to their seed production systems. In a molecular characterization of 330 rice varieties collected from 59 villages in Burkina Fasso with 23 SSRs, Kam et al. (2017) were able to separate the varieties belonging to the species *O. sativa* and *O. glaberrima* and those resulting from the probable natural crossing of the two species. The varieties of *O. glaberrima* were divided into two subgroups according to their culture ecosystem (submersion and lowland) and similarly. Moreover, those of *O. sativa* were classified into three subgroups according to their late maturity in lowlands, small size and early maturity adapted to lowland conditions as well as their large size and late maturity adapted to submerged conditions. Our results agree with those from Kam et al. (2017) but slightly different based on the presence of interspecific rice varieties and other unknown genetic origin rice varieties under group II. Principal Component Analysis (PCA) showed that all the varieties were classified into three groups with a few isolated genotypes (Figure 3).

Groups I and III comprised interspecific varieties adapted to rainfed rice cultivation and varieties of unknown genetic origin, respectively. In contrast, group II consisted of a mixture of varieties from *O. sativa, O. glaberrima, O. longistaminata*, and interspecific hybrids. This classification differs from that obtained in the dendrogram of Figure 2 and from Kam et al. (2017), who identified groups comprising *O. glaberrima, O. sativa* japonica, *O. sativa indica*, interspecific varieties, and others without precise classification. However, our grouping aligns with Chen et al. (2017), who distinctly grouped varieties of *O. sativa, O. glaberrima*, and interspecific (NERICA), with two *O. sativa* varieties and NERICA isolated.

The percentage of explained variation by the two principal components was 12.58 and 7.64%, respectively. The grouping of rice genotypes depends on the affinity of the microsatellite markers used for diversity assessment, which may be related to the subspecies of *O. sativa*. For instance, Hour et al. (2020) conducted a diversity study of local and improved varieties of *O. sativa* in Taiwan, revealing two large distinct groups corresponding to the subspecies *indica* and *japonica*. They also observed a clear separation between local subspecies and cultivars.

Conclusion

The molecular characterization of lowland and upland rice varieties in Mali has revealed significant genetic diversity. Genetic parameters varied among varieties, distinguishing them based on their cultivation ecosystem and genetic origin. Some varieties collected from producers formed distinct genetic groups, separate from interspecific varieties used in lowland and upland rice cultivation, while others closely resembled these interspecific varieties used in lowlands. These similarities suggest potential natural crossings between *O. sativa* and other species. These findings can assist breeders in identifying potential parents with desirable agronomic traits such as high yield, early maturity, and a high number of tillers. These parents could be utilized in breeding programs aimed at improving varieties favored by farmers, which may currently exhibit low yields, late maturity, and a low number of tillers.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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