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Comparative genetic diversity *between Oryza sativa, Oryza glabberima,* and interspecific hybrid (NERICA) based on SSR markers

Ramatu E. Aliyu[1*], Sadam S. Indabo[2], Hauwa O. Ahmed[3], Aminu Aliyu[1], Hadiza U. Muhammad[2], Solihu K Sakariyahu[2] and Abu-Kassim Adamu[1]

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[1]Department of Botany, Faculty of Life Sciences, Ahmadu Bello University, Zaria
[2]Department of Biology, Faculty of Life Sciences, Ahmadu Bello University, Zaria
[3]Department of Plant Science, Faculty of Agriculture, Ahmadu Bello University, Zaria

***Corresponding author's email:**

s.ramatu@gmail.com;

enehezeyi@abu.edu.ng

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A comprehensive molecular analysis of 110 rice genotypes, comprising *Oryza sativa, Oryza glaberrima,* and the interspecific hybrid NERICA, was conducted using 34 microsatellite markers. This assessment revealed an extensive genetic diversity, with 3,866 polymorphic bands (95.1%) and 12 rare alleles (9.8%) identified across the polymorphic microsatellite loci. Notably, *O. glaberrima* and NERICA exhibited a significantly higher frequency of genotypes with rare alleles (12.5%) compared to *O. sativa*. A total of 123 alleles were detected, with an average of 3.62 alleles per locus, ranging from 2 to 7 alleles per locus. The allelic distribution varied among genotypes, with *O. glaberrima* displaying the lowest allelic number of 4 at locus RM182. The polymorphic information content (PIC) value averaged 0.56 per marker, with a range of 0.16 (RM182) to 0.78 (RM547), indicating a high level of genetic diversity among the rice genotypes. The PIC (Polymorphic Information Content) values for NERICA and *O. glaberrima* were calculated to be 0.51 and 0.37, respectively, indicating moderate to high genetic diversity. Cluster analysis revealed a clear grouping of rice genotypes into eight distinct clusters at a similarity coefficient of 68%, with genotypes sharing similar genetic backgrounds co-clustering. Notably, *O. sativa* exhibited significantly higher genetic diversity compared to O. glaberrima. These findings suggest that the genetic base of *O. glaberrima* can be expanded through the incorporation of novel alleles, which can be leveraged to introduce desirable traits in breeding programs, thereby enhancing the genetic potential of this species.

Keywords: Genetic diversity; Nerica.; *O. glaberrima; O. sativa;* SSR markers; Rare alleles.

1. Introduction

The genomic relationship among *Oryza*, a genus of great agricultural importance with a basic chromosome number of twelve, has been explored through multiple types of genetic and cytogenetic analyses. These analyses include chromosome pairing (Katayama, 1982), morphology (Morishima and Oka, 1970), isozymes (Glaszman, 1987), and DNA polymorphism (Wang *et al.*, 1992; Aggarwal *et al.*, 1999; Ge *et al.*, 1999; Ren *et al.*, 2003; Aliyu *et al.*, 2023). The wild relatives of cultivated rice are important constituents of the rice gene pool and have significantly contributed to breeding programmes (Muhammad *et al.*, 2024). The cultivated Asian rice species, *O. sativa*, is composed of two subspecies: Indica and Japonica (Oka, 1988). Indica is the main tropical subspecies, while Japonica, which has lower genetic diversity (Glaszman, 1987; Zhang *et al.*, 1992), grows widely in East Asia, South America, Australia, the Mediterranean, North Africa, and Europe. Japonica accounts for approximately 20% of the world rice population (Mackill, 1995).

Oryza sativa and *O. glaberrima* are both AA genome species with minor sub-genomic differences. They evolved through independent and parallel evolutionary processes in Asia and Africa, respectively (Ghesquire *et al.*, 1996). *Oryza glaberrima*, a cultivated rice species endemic to Africa, possesses useful traits that can enhance the productivity of *O. sativa* cultivars (Sarla and Mallikarjuna, 2005). However, F_1 hybrids between these two species result in complete sterility, regardless of the parental varieties used (Chang, 1976; Pham and Bougerol, 1993). This sterility may be caused by reduced recombination and distorted segregation due to wide hybridization,

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making the selection of desired recombinants during the breeding process difficult (Pham and Bougerol, 1993).

Understanding genetic diversity and the extent of species interrelationships in wild plant populations is essential for elucidating the process of evolution (Frankel and Bannet, 1970). This knowledge is crucial for developing effective strategies for the collection and conservation of plant populations (Frankel and Bannet, 1970) and for the introgression of useful genes from wild relatives into cultivated crops (Soon-Jae *et al*., 2008). Plant breeders commonly apply these practices, using wild relatives as donors of important traits within the same subspecies or cultivar group. Selection can be enhanced through molecular marker technologies, which are reliable, efficient, and valuable tools for analysing genetic relatedness and identification. This is because diversity based on morphological and phenological characters often varies with the environment, and evaluating these traits requires growing the plants to maturity before identification.

Simple Sequence Repeat markers (microsatellites) are co-dominant, highly variable, and suitable for high-throughput analysis (Kumpatla, 2008), and they are evenly distributed throughout the genome (Temnykh *et al*., 2001). They have been shown to be polymorphic both between (Akagi *et al*., 1997; Chen *et al*., 1999) and within rice populations (Olufowote *et al*., 1997). SSRs are extensively utilized as molecular markers for fingerprinting germplasm, assessing genetic diversity, conducting pedigree analysis, studying evolutionary processes, and mapping genomes (Yang *et al*., 1994; McCouch *et al*., 1997; Gerlant *et a*l., 1999). They are also valuable for optimizing the assembly of core collections (Shoen and Brown, 1995).

We hypothesize that the African rice gene pool is still underutilized. The objectives of this study were to evaluate genetic variation and diversity among rice genotypes using microsatellite markers, to determine differences in diversity patterns among and within species, and to provide information to breeders about genotypes with rare alleles that could be used in cultivar advancement.

2. Materials and Methods

2.1 Plant material

A collection of 92 *O. sativa* (both japonica and indica), 15 Nerica (*O. sativa* × *O. glaberrima*), and 3 *O. glaberrima* rice seeds were obtained from the rice germplasm collections at the International Rice Research Institute (IRRI) in the Philippines, the Africa Rice Centre (AfricaRice) in Nigeria, and the International Institute of Tropical Agriculture (IITA) in Ibadan, Nigeria.

2.2 DNA extraction and SSR markers

Genomic DNA was extracted from young leaf tissues following the method described by Dellaporta *et al*. (1983), with modifications (Aliyu *et al*., 2017), using 5 mg of leaf material from at least 3 seedlings. Thirty-four (34) polymorphic rice microsatellite markers (Table 1) were used in this study. The original sources and motifs for these markers were reported by Temnykh *et al*. (2000) and the rice gene database (http://ars.genome.cornell.edu/rice), as verified on October 2, 2001. The nucleic acid concentration was quantified using an ND-1000 spectrophotometer (NanoDrop Technologies Inc., 2007; www.nanodrop.com).

2.3 Polymerase chain reaction

Polymerase chain reaction (PCR) was conducted in a 10 µL reaction mixture containing 1× PCR buffer, 1 µL of 25 mM $MgCl_2$, 0.2 µL of 10 mM dNTPs, 0.25 µL of 20 µM primer pairs, 0.5 µL of Taq polymerase, 2.15 µL of Milli-Q water, and 5 µL of 10 ng/µL DNA, using a Perkin-Elmer thermocycler according to the following cycle profile: (i) preheating at 94°C for 2 minutes, (ii) denaturation at 94°C for 30 seconds, followed by 34 cycles of 30 seconds at 94°C, 30 seconds annealing at 55°C or 67°C (depending on the marker used), and 30 seconds initial extension at 72°C, (iii) final extension at 72°C for 2 minutes.

The amplified products were subjected to electrophoresis in a 2% agarose gel in 1× TBE buffer at 78-80 volts for 2-3 hours for optimal separation. The gel was stained with 0.5 µL of ethidium bromide for 5 minutes and visualized under ultraviolet light using a gel documentation system.

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2.4 Data analysis

2.2.1 Polymorphic Information Content (PIC).

The value of a marker for detecting polymorphism between genotypes for each primer combination, based on the number of detectable alleles and the distribution of their frequencies, was calculated using the formula proposed by Anderson *et al.* (1993).

$$PIC_i = 1 - \sum_{J-1}^{n} Pij2$$

Where Pij is the frequency of the j-th allele for marker iii, and the summation extends over n alleles. The number of alleles per locus was based on an evaluation of 110 rice genotypes using 34 SSR markers (Table 2).

2.2.2 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 repeat numbers of amplified fragments were treated as a unit character and scored as a binary code (1 for presence and 0 for absence). Only prominent and unambiguous bands were scored to ensure data reliability. Genetic similarities were estimated from the matrix of binary data using the Jaccard similarity coefficient for cluster analysis, according to the Unweighted Pair Group Method with Arithmetic Mean (UPGMA), and dendrograms were constructed to show the relatedness of the genotypes using NTSYS software (Rolph, 2002). The data were further analysed using DARWIN software (Perrier and Jacquemond, 2006).

3. Results and Discussion

3.1 SSR polymorphism

A total of 3,866 (95.1%) polymorphic bands were detected at 123 microsatellite loci evaluated across 110 genotypes using 34 polymorphic SSR markers. The number of alleles ranged from 2 (RM17, RM216, RM480, RM495, RM514, RM518, and RM527) to 7 (RM481), with an average of 3.6 alleles per locus and amplicon sizes ranging from 110 bp to 340 bp for RM286 and RM340, respectively. The average number of alleles per locus was within the range reported by Cho *et al.* (2000) but was not comparable to the average of 5.89 and 6.8 alleles per locus reported by Lapitan *et al.* (2007) in their study of the genetic diversity of Philippine rice cultivars and Mackill *et al.* (2002) in their study of 38 rice cultivars in U.S. breeding programmes. However, microsatellites have been identified to produce significantly greater allelic diversity than other molecular markers (McCouch *et al.*, 2001). Among the 34 primers, fingerprinting of *O. glaberrima* can be done with an allele at RM483 and RM481, while two alleles at RM584 and one allele at RM528 were diagnostic for Nerica, as DNA fingerprinting facilitates germplasm characterization (Chakravarthi and Naravaneni, 2006).

3.2 Comparative SSR polymorphism

An assessment of genetic diversity among *Oryza sativa* genotypes, comprising both japonica and indica subspecies, revealed a high degree of polymorphism at 119 microsatellite loci using a panel of 34 microsatellite markers. Allelic variation ranged from 2 to 6 alleles per locus, with loci RM17, RM216, RM495, RM514, RM518, and RM527 exhibiting the lowest allelic diversity (2 alleles), while loci RM481, RM474, RM547, and RM562 displayed the highest allelic diversity (6 alleles). The average allelic richness per locus was 4.2, accounting for 35% of the total allelic repertoire and contributing 43% to the overall allelic frequency per genotype**.** Nerica had 103 polymorphic alleles, ranging from 1 (RM480) to 5 (RM547), with an average of 3.7 alleles per locus, contributing about 31% of the allelic frequency per genotype. *Oryza glaberrima* had 53 alleles and an allelic frequency of 26%, with alleles ranging from 1 (RM1, RM17, RM21, RM216, RM286, RM320, RM474, RM480, RM488, RM508, RM514, RM539, RM547, RM584, and RM587) to 4 (RM182), with an average of 1.6 alleles per locus. This was much lower than the values reported by Semon *et al.* (2005) using RFLP markers over a larger accession size.

The average number of alleles per locus observed in *Oryza sativa* and Nerica was consistent with those reported in previous studies using other types of markers, such as isozymes (Glaszmann, 1987; Second, 1982) and RFLPs (Wang and Tanksley, 1989; Zhang *et al.*, 1992). However, the average number of alleles was less than those reported by Yang *et al.* (1994), who observed an average of 9.3 alleles at one locus, and Jain *et al.* (2004), who recorded 7.8 alleles per locus with a range of 3-22 using Indian aromatic and quality rice germplasm accessions, and by Lapitan *et al.* (2007), who used 151 polymorphic markers.

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Asian *Oryza sativa* genotypes have a higher level of polymorphism compared to African *Oryza sativa* genotypes. This result is consistent with reports by Aggarwal *et al.* (1999) using AFLP, Park *et al.* (2003) using MITE-TD, and Ishii *et al.* (2002) using SSR markers. The high level of polymorphism recorded for *Oryza sativa* may be attributed to its wide distribution (Vaughan, 1994), as selection increases the frequency of alleles or allelic combinations with favourable effects at the expense of others, eventually eliminating them (Coa *et al.*, 1993). Across the genotypes, *Oryza sativa* did not share 20 (16.8%) alleles with Nerica and 70 (58.8%) alleles with *Oryza glaberrima*, indicating that Nerica is closer to *Oryza sativa* than *Oryza glaberrima*.

3.3 Rare Alleles

Microsatellite analysis revealed a diverse array of allelic frequencies among the genotypes, with 111 alleles exhibiting high frequencies (≥ 0.05) across 30 loci. The allelic frequency spectrum ranged from 98% (RM84) to 6% (RM481), indicating varying degrees of genetic variation. Notably, 12 rare alleles (9.8% of 123 polymorphic loci) were identified, with RM528, RM481, RM528, and RM584 each harboring two rare alleles. Furthermore, RM84, RM481, and RM483 were associated with the highest number of genotypes (five) carrying rare alleles, followed by RM562 with four genotypes. These findings highlight the genetic diversity and allelic variation present in the studied genotypes. The genotypes CG14 (*O. glaberrima*), Nerica U-7, and Nerica U-3 exhibited the highest number of rare alleles across the polymorphic loci, followed closely by TOG 5602 and TOG 5270 (O. glaberrima) with three rare alleles each. Notably, the *O. sativa* hybrid IR77674-3B-8-1-10-4 possessed rare alleles at two loci, while LAC 23, an African *O. sativa* genotype utilized for local cultivar improvement, displayed rare alleles at two loci corresponding to *O. glaberrima* and upland Nerica. This finding suggests an admixture of *O. glaberrima* with *O. sativa*, consistent with previous reports (Semon *et al.*, 2005). The presence of rare alleles in these genotypes underscores their significance as a valuable resource for plant breeders and geneticists, offering a rich source of genetic material for potential exploitation in breeding programs.

3.4 Haplotypes among *O.glaberrima* and Nerica

The *Oryza glaberrima* genotypes examined exhibited haplotypic uniformity at 13 (38%) of the polymorphic markers, indicating a limited genetic diversity and underutilization of this species. Interestingly, these genotypes shared a higher number of alleles with upland Nerica than with lowland Nerica (Table 3), likely due to the fact that CG14, an *Oryza glaberrima* genotype, is one of the parents of upland Nerica.

3.5 PIC value

The mean polymorphic information content (PIC) value across all markers was 0.56, ranging from 0.16 (RM182) to 0.78 (RM547), with RM547 being the most informative marker, followed by RM539 and RM562 (Table 2). The PIC values for Nerica and *O. glaberrima* were 0.51 and 0.37, respectively, with Nerica exhibiting a range of 0.09 (RM231) to 0.81 (RM474), and O. glaberrima showing a range of 0.14 (RM9) to 0.78 (RM72, RM501, and RM584). The low PIC value observed in *O. glaberrima* is consistent with previous reports (Semon *et al.*, 2005), highlighting the need for outcrossing to enhance genetic variation within this species.

3.6 Cluster analysis

A dendrogram generated using UPGMA and Jaccard similarity coefficient revealed 8 major groups of genetically similar rice genotypes at a 68% similarity threshold (Fig. 1). Group 1 consisted of a single Indian lowland *O. sativa* genotype with a salt-tolerant gene. Group 2 (69% similarity) comprised 10 genotypes, including 7 Asian *O. sativa* and 3 improved African *O. sativa*. Group 3 (69% similarity) had 3 clusters, including lowland Nerica, African and Asian *O. sativa*, and Asian *O. sativa*. Group 4 (75% similarity) consisted of 6 *O. sativa* genotypes, potentially developed from the same parent. Group 5 comprised Asian *O. sativa* hybrids, with IR77674-3B-8-1-1-10-4 forming an outlier cluster. Group 6 consisted of Asian *O. sativa* genotypes, including moderately salt-tolerant FL 478. Group 7 comprised 3 genotypes: TOX 4004-43-1-2-3, Nerica L-41, and BOUAKE 189. Group 8 had 2 clusters: the first cluster of 13 genotypes comprised upland and lowland Nerica and Asian *O. sativa*, indicating shared ancestry. The second sub-cluster consisted of 4 improved African *O. sativa* genotypes. The second cluster had 2 sub-clusters. The first sub-cluster comprised *O. glaberrima* genotypes (TOG 5601, TOG 5670, and CG14) at an 81% similarity coefficient. The second sub-cluster consisted of 3 upland Nerica genotypes. The second cluster, at a 75% similarity coefficient, comprised 2 upland Nerica and an outlier of an African *O. sativa* genotype (ITA 212), which possibly shares

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common alleles with the upland Nerica and could be used for breeding purposes.

A dendrogram (Fig. 2) analyzing allelic similarity among Nerica and *O. glaberrima* revealed 5 major clusters at 62% similarity. Clusters 1 and 5 comprised lowland Nerica genotypes, while Cluster 2 had sub-clusters of upland Nerica and *O. glaberrima* genotypes sharing common alleles and haplotypes. Cluster 3 consisted of upland Nerica genotypes with rare alleles. Nerica L-41 clustered separately, suggesting closer relation to *O. sativa*. Radial phylograms (Fig. 3A and 3B) and PCA (Fig. 4) confirmed genetic relationships, with genotypes from similar types clustering together. This spatial structure of genetic variation is consistent with previous reports (Ge *et al*., 1999; Joshi *et al*., 1999; Ren *et al*., 2003). The low diversity within *O. glaberrima* may be attributed to environmental factors (Buso *et al*., 1998), while varying diversity levels may result from mutation rates, migration, and selection intensities influenced by location, climate, and soil (Nevo *et al*., 1979; Frankel, 1984; Parsons *et al*., 1997; Kork *et al*., 1999).

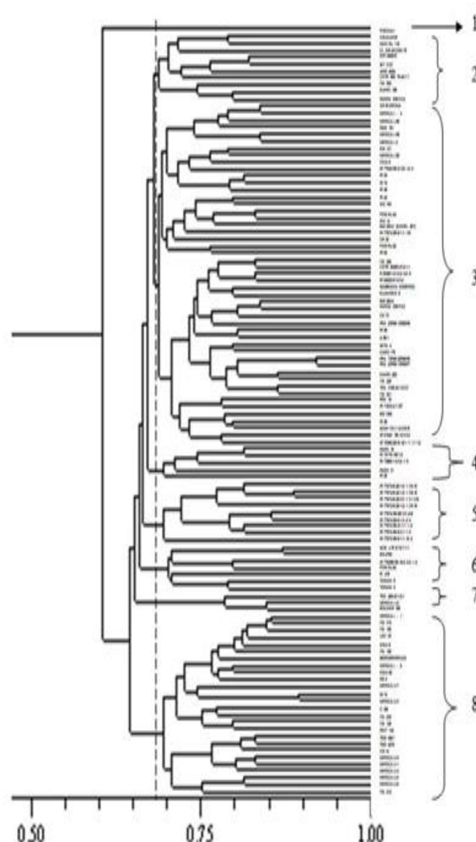


Fig. 1. Dendrogram showing the genetic diversity of 110 rice genotypes based on 34 polymorphic SSR markers derived from, UPGMA cluster analysis using NTSYS (Jaccard coefficient).

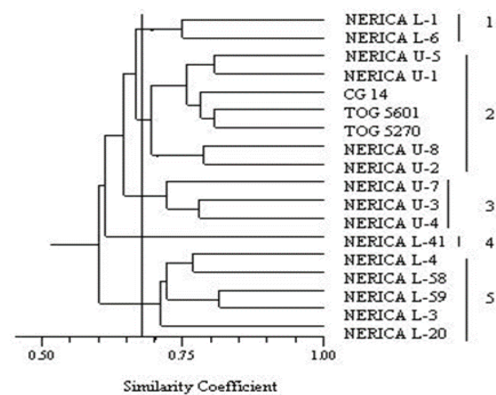
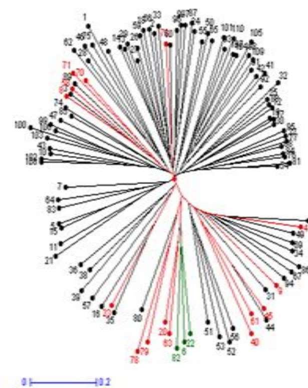


Fig. 2: Dendrogram showing the genetic diversity of NERICA genotypes based on 34 polymorphic SSR markers derived from, UPGMA cluster analysis using NTSYS (Jaccard coefficient)



(A)

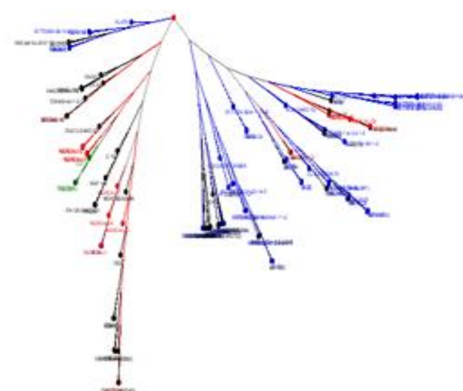


Fig. 3. (A) Radial phylogram (neighbor joining) of 110 rice genotypes (*O. sativa* – black, *O. glaberrima*- green, Interspecifics hybrid – red) showing genetic diversity based on 34 SSR markers. (B) Rooted phylogram representing shared allele frequencies among 110 rice genotypes; *O. glaberrima* are shown in green; *O. sativa* x *O. glaberrima* are shown in red; and *O. sativa* (African origin*)* are shown in black, *O. sativa* (Asian origin) are shown in blue. Perrier X, Jacquemoud – Collet J.P (2006) DARwin software.

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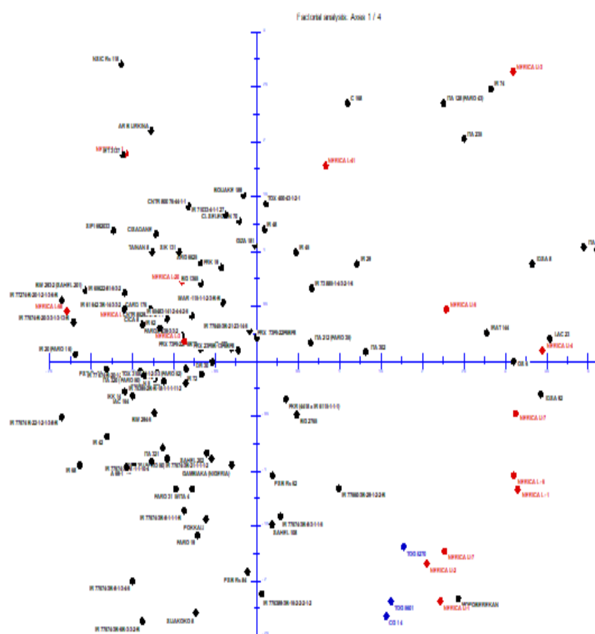

Fig 4. Principal coordinate analysis showing distance partitioning from the originate of 110 of rice genotypes (*O. glaberima*- blue, Interspecifics- red, *O. sativa* – black). Perrier X, Jacquemoud – Collet J.P (2006) DARwin software.

Table 2. Data on number of alleles and polymorphic information content (PIC) value of 34 SSR markers 0f 110 rice genotypes.

Marker Accession	Allele no	PIC Value
RM 1	3	0.61
RM9	5	0.30
RM17	2	0.50
RM21	3	0.68
RM72	4	0.73
RM84	3	0.21
RM182	5	0.16
RM216	2	0.53
RM231	3	0.37
RM240	4	0.33
RM286	4	0.67
RM287	3	0.76
RM315	3	0.54
RM320	4	0.71
RM407	3	0.52
RM474	6	0.72
RM480	2	0.32
RM481	7	0.68
RM483	3	0.55
RM488	3	0.55
RM495	2	0.43
RM501	4	0.64
RM508	3	0.56
RM514	2	0.49
RM518	2	0.48
RM520	3	0.69
RM527	2	0.57
RM528	6	0.49
RM539	3	0.77
RM547	6	0.78
RM562	6	0.77
RM584	5	0.68
RM587	4	0.61
RM600	3	0.54
Mean	3.62	0.56

Table 3: Haplotypes produced within Nerica with 13 SSR markers with reference to *0. glaberrima.*

RM1 RM17 RM21 RM216 RM286 RM483 RM488 RM495 RM508 RM514 RM518 RM539 RM587

NERICA L-1
NERICA L-3
NERICA L-4
NERICA L-6
NERICA L-20
NERICA L-41
NERICA L-58
NERICA L-59
NERICA U-1
NERICA U-2
NERICA U-3
NERICA U-4
NERICA U-5
NERICA U-7
NERICA U-8
CG 14
TOG 5270
TOG 5601

4. Conclusion

The molecular profiling of rice genotypes using DNA markers revealed significant genetic diversity and relationships within and among *O. glaberrima,* Nerica, and *O. sativa*. The identification of specific markers for *O. glaberrima* and Nerica enables accurate gene mapping, informing the development of new varieties, improvement of local cultivars, and investigations into the evolutionary history of the rice genus. The results suggest that *O. glaberrima* and Nerica possess unique genetic resources that can be leveraged for rice improvement, and their relationships with *O. sativa* provide opportunities for gene introgression and crop enhancement. Overall, this study contributes to the understanding of rice genetic diversity and provides valuable tools for rice breeding and conservation programs.

Conflict of interest

The authors declare no conflict of interest.

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Table 1. Location, repeat motif and primer sequence of 34 polymorphic SSR primers used to amplify rice genotypes in this study

Primers	Chrom	Repeat Motif	Primer Sequence (Forward)	Primer Sequence (Reverse)
RM 1	1	(GA)26	GCGAAAACACAATGCAAAAA	GCGTTGGTTGGACCTGAC
RM9	1	(GA)15GT(GA)2	GGTGCCATTGTCGTCCTC	ACGGCCCTCATCACCTTC
RM17	12	(GA)21	TGCCCTGTTATTTTCTTCTCTC	GGTGATCCTTTCCCATTTCA
RM21	11	(GA)18	ACAGTATTCCGTAGGCACGG	GCTCCATGAGGGTGGTAGAG
RM72	8	(TAT)5C(ATT)15	CCGGCGATAAAACAATGAG	GCATCGGTCCTAACTAAGGG
RM84	1	(TCT)10	TAAGGGTCCATCCACAAGATG	TTGCAAATGCAGCTAGAGTAC
RM182	7	(AT)16	TGGGATGCAGAGTGCAGTTGGC	CGCAGGCACGGTGCCTTGTAAG
RM216	10	(CT)18	GCATGGCCGATGGTAAAG	TGTATAAAACCACACGGCCA
RM231	3	(CT)16	CCAGATTATTTCCTGAGGTC	CACTTGCATAGTTCTGCATTG
RM240	2	(CT)21	CCTTAATGGGTAGTGTGCAC	TGTAACCATTCCTTCCATCC
RM286	11	(GA)16	GGCTTCATCTTTGGCGAC	CCGGATTCACGAGATAAACTC
RM287	11	(GA)21	TTCCCTGTTAAGAGAGAAATC	GTGTATTTGGTGAAAGCAAC
RM315	1	(AT)4(GT)10	GAGGTACTTCCTCCGTTTCAC	AGTCAGCTCACTGTGCAGTG
RM320	7	(AT)11GTAT(GT)13	CAACGTGATCGAGGATAGATC	GGATTTGCTTACCACAGCTC
RM407	8	(AG)13	GATTGAGGAGACGAGCCATC	CTTTTTCAGATCTGCGCTCC
RM474	10	(AT)13	AAGATGTACGGGTGGCATTC	TATGAGCTGGTGAGCAATGG
RM480	5	(AC)30	GCTCAAGCATTCTGCAGTTG	GCGCTTCTGCTTATTGGAAG
RM481	7	(CAA)12	TAGCTAGCCGATTGAATGGC	CTCCACCTCCTATGTTGTTG
RM483	8	(AT)26	CTTCCACCATAAAACCGGAG	ACACCGGTGATCTTGTAGCC
RM488	1	(GA)17	CAGCTAGGGTTTTGAGGCTG	TAGCAACAACCAGCGTATGC
RM495	1	(CTG)7	AATCCAAGGTGCAGAGATGG	CAACGATGACGAACACAACC
RM501	7	(TC)10(TA)21	GCCCAATTAATGTACAGGCG	ATATCGTTTAGCCGTGCTGC
RM508	6	(AG)17	GGATAGATCATGTGTGGGGG	ACCCGTGAACCACAAAGAAC
RM514	3	(AC)12	AGATTGATCTCCCATTCCCC	CACGAGCATATTACTAGTGG
RM518	4	(TC)15	CTCTTCACTCACTCACCATGG	ATCCATCTGGAGCAAGCAAC
RM520	3	(AG)10	AGGAGCAAGAAAAGTTCCCC	GCCAATGTGTGACGCAATAG
RM527	6	(GA)17	GGCTCGATCTAGAAAATCCG	TTGCACAGGTTGCGATAGAG
RM528	6	(AGAT)9	GGCATCCAATTTTACCCCTC	AAATGGAGCATGGAGGTCAC
RM539	6	(TAT)21	GAGCGTCCTTGTTAAAACCG	AGTAGGGTATCACGCATCCG
RM547	8	(ATT)19	TAGGTTGGCAGACCTTTTCG	GTCAAGATCATCCTCGTAGCG
RM562	1	(AAG)13	CACAACCCACAAACAGCAAG	CTTCCCCCAAAGTTTTAGCC
RM584	6	(CT)14	AGAAAGTGGATCAGGAAGGC	GATCCTGCAGGTAACCACAC
RM587	6	(CTT)18	ACGCGAACAAATTAACAGCC	CTTTGCTACCAGTAGATCCAGC
RM600	1	(TTA)19	AAACGTGTGTTAGCCTGTTAGG	CATATGCTAGTGGTGCTAGCG

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