



Research Article

Genetic Diversity among Selected Nigerian Rice (*Oryza sativa* L.) Cultivars for Agronomic Improvement Towards Sustainable Food Security

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ABSTRACT

Rice (*Oryza sativa* L.) is an important cereal crop which serves as a staple food for millions of people in Nigeria and more than 50% of the world's population. Therefore, with the changing climate and global food challenges, the need to identify potential rice (*Oryza sativa* L.) cultivars, cannot be over emphasised. Hence, the study seeks to explore and measure the level of genetic diversity among selected rice germplasm resources in Nigeria, to identify potential candidates for rice improvement programs. The experimental layout was a complete randomized block design (CRBD) with three replicates per cultivar. Genetic diversity was assessed using 10 SSR markers on 40 Nigerian rice cultivars. The study revealed significant variability among evaluated cultivars. Cluster analysis using molecular data divided the 40 cultivars into two major clusters with sub clusters. Molecular analysis revealed 3.8 average alleles, heterozygosity ($H_e = 0.55$), gene diversity ($G_D = 0.58$), Shannon's index ($I = 0.49$) and PIC of 0.51 among rice cultivars indicating a moderate level of genetic diversity. Heritability was highest in grain yield ($H^2 = 0.97$). The observed polymorphism suggests potential cultivars with ample opportunity for crop improvement program to develop new resilient and high yielding climate smart cultivars through breeding.

Keywords: Cultivar; Diversity; *Oryza sativa*; Rice; Variation

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INTRODUCTION

Rice (*Oryza sativa* L.) is a major cereal crop with over 50% of the world's population depending on it for their daily requirements, accounting for more than 20% of calories consumed worldwide. Nigeria is currently among the major producers and consumers of rice in Africa (Bin Rahman and Zhang, 2023). However, Nigerian rice farmers are still faced with several challenges which include scarcity of disease-resistant and high-yielding cultivars adaptable to adverse weather conditions. Despite the production rate of rice in Nigeria, the country remains among the major importers of rice consequently draining the

nations' foreign exchange reserves and posing threat to national food security. Therefore, additional study is required to determine potential accessions, varieties that may be selected for crop improvement in this agronomically and commercially significant crop.

The genetic diversity within germplasm resources is a major feature in rice breeding advancement. Hence, the knowledge of the variation within the germplasm resources would help enhance rice production in Nigeria (Suvi *et al.*, 2020). Several studies assessing the genetic and phenotypic diversity of rice genotypes have been carried out (Suvi *et al.*, 2020;

Ndjiondjop *et al.*, 2022; Kimwemwe *et al.*, 2023). However, there is a dearth of information on the performance and diversity of Nigerian rice cultivars. Nigeria holds several rice cultivars; however, the development of these cultivars was mostly driven by selection based on agronomic qualities by the farmers, with little regard for genetic diversity. Therefore, this study seeks to assess the level of diversity and performance among selected rice cultivars in Nigeria to identify potential germplasm for agronomical improvement.

MATERIALS AND METHODS

Collection of samples

A total of 40 different varieties of Nigerian rice seed were investigated. The seeds were collected from National Cereals Research Institute, Baddegi, Niger State. These samples were planted at the Department of Botany screen house, University of Lagos, Nigeria (Latitude 6° 31' 01.2'' N, Longitude 3° 23' 30.9'' E).

Cultivation of Nigerian rice cultivars

Seven seeds were sown into pre-wet soil per pot, with each plant row consisting of six potted plants spaced 0.15 m apart, with an inter-row spacing of 0.40 m apart in a complete randomized block design layout. Irrigation commenced immediately after seeds were sown and continued throughout the cultivation period. At day 14 after seedling emergence, the germinated seedlings were thinned down to three per pot. The first weeding was carried out manually alongside the thinning down of the germinated rice varieties, while the second weeding was carried out during panicle initiation.

Molecular characterization

Extraction of genomic DNA from Nigerian rice cultivars

Genomic DNA was extracted from the young fresh leaves collected from each rice cultivar following the manufacturers’ protocol of the standard Zymo Plant/Seed DNA extraction kits. The concentration and purity of the extracted DNA were checked using the Nanodrop Spectrophotometer (UV5Nano, Mettler-Toledo GmbH, Switzerland), while the quality was verified using gel electrophoresis in a 0.5× TBE buffer.

Polymerase Chain Reaction

Ten different pairs of SSR primers (Inqaba Biotec, West Africa Limited) were used for the assessment of genetic diversity among 40 sampled extracted DNA. All the primers yielded clear amplification bands that were used for the diversity analysis. The PCR was done in a 10 µL reaction volumes containing 1.0 µL template DNA, 2µL 5× FIREPol® Master Mix (FIREPol® DNA polymerase, 0.4 M Tris-HCl, 0.1 M (NH₄)₂SO₄, 0.1% w/v Tween-20, 12.5 mM MgCl₂, 200 µM dATP, 200 µM dCTP, 200 µM dGTP, 200 µM dTTP), 0.5 µL each of forward and reverse primers, and 6.0 µL nuclease-free water. PCR amplification was done in a thermal cycler (A100 Gradient Thermal Cycler, Long Gene, China). Initial denaturation was performed at 94°C for 10 min, followed by 40 cycles of 30 s denaturation at 94°C, then, 1 min 30 s annealing at 58°C, and 1 min 30 s extension at 72 °C. The final extension was done at 72 °C for 10 min. The amplified products were resolved in 2% agarose with 0.5× TBE buffer stained with 10 µL of ethidium bromide (10 mg/mL). The setup was run at 70 V for 1 h 30 min. Gel documentation was done in an ultraviolet Trans-illuminator documentation system (UVIDOC HD6, UVITEC, Cambridge, UK).

Table 1. Primer ID and sequences used in the study

Primer ID	Forward sequence (5' – 3')	Reverse sequence (5' – 3')
M-06	AGGAATGGCTCAATACAT	AGAAAGCAGTTGGATTGGT
M-09	AATGCCACCTGAGATTTATG	ACCTAATTCTCCAGCTCAA
M-14	CGAGACCTGATTTGTTTAGC	CAAGTCTTTGATTTCCGTCT
M-19	CATCTTCGAGGTTCTTGGT	AGCAGTGATTCGGTAGGA
M-26	AATACATTTTCCCTCCGTC	GGATCTCGTTCATGTGCTAT
M-28	GCTGGACTCTGAAGTGGATA	AATCCTAGTTATGGGCGTTC
M-30	GTGCTAGGTGGAGCGAGA	CGTGAGCAGGTTCTCCAG
M-31	TTAAGACCATTTGGATTAGAGAA	CTTAAACGCCAATCTTTAG
M-32	ATGAGGTGAAGCAGAGGAG	CGTGGACTAACTCAACAACAAGG
M-34	ACTAACACCAGCGGTGA	CTAGCAGTGTTCATGTGC

Analysis of Molecular Data

The molecular data were scored manually using the binary coding system, where clear DNA bands were scored '1' for the presence of a band and '0' for the absence of a band for each primer. The bands that were not polymorphic with at least one of the samples were not scored. The molecular data matrix obtained was used to construct a dendrogram based on Unweighted Pair Group Mean with Arithmetic (UPGMA) and dissimilarity index in jaccard's option (Ojuedri et al. 2013) Molecular data analysis was done using GenAlex 6.5 software (Peakall & Smouse, 2006, 2012 version).

RESULTS

Marker performance and diversity analysis among the Nigerian rice cultivars

The results revealed that SSR markers used on the sampled rice cultivars gave multiple alleles per marker ranging from 2 alleles (in M26 and M34) to 7 alleles (in M30), with an average value of 3.80 alleles in the study (Table 1). These markers were able to show a level of heterozygosity between 0.15 (M06) and 1.00 (M30), with a mean heterozygosity of 0.55. Gene diversity was highest with DE30 (M = 0.84) and least with M14 (GD = 0.14). Shannon's index was highest with M26 (I = 0.62), and least with M06 (I = 0.36), with a mean of 0.49. The average PIC for the markers used in the study was 0.51 (Table 2).

Table 2. Marker characteristics within Nigerian rice cultivars evaluated

Marker	Na	GD	He	I	PIC
M06	4	0.52	0.15	0.36	0.44
M09	3	0.62	0.19	0.41	0.54
M14	3	0.35	0.29	0.38	0.31
M19	4	0.55	0.72	0.43	0.51
M26	2	0.47	0.63	0.65	0.36
M28	5	0.77	0.76	0.45	0.73
M30	7	0.84	1.00	0.62	0.82
M31	3	0.47	0.29	0.47	0.38
M32	5	0.77	0.76	0.51	0.73
M34	2	0.41	0.52	0.63	0.33
Mean	3.8	0.58	0.55	0.49	0.51

Na: number of alleles, GD: gene diversity, He: Heterozygosity, I: Shannon index, PIC: polymorphic information content

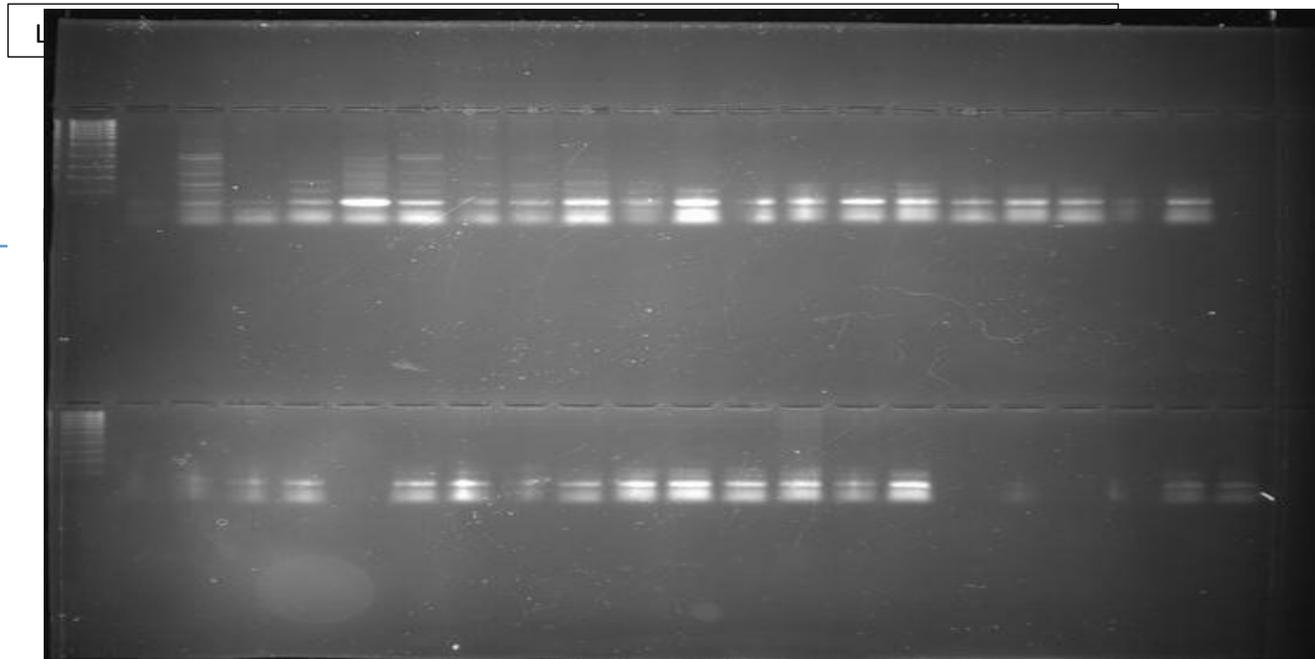


Plate 1. Polymorphic bands showing the amplicons from 1-40 cultivars PCR amplified by M-28 primers

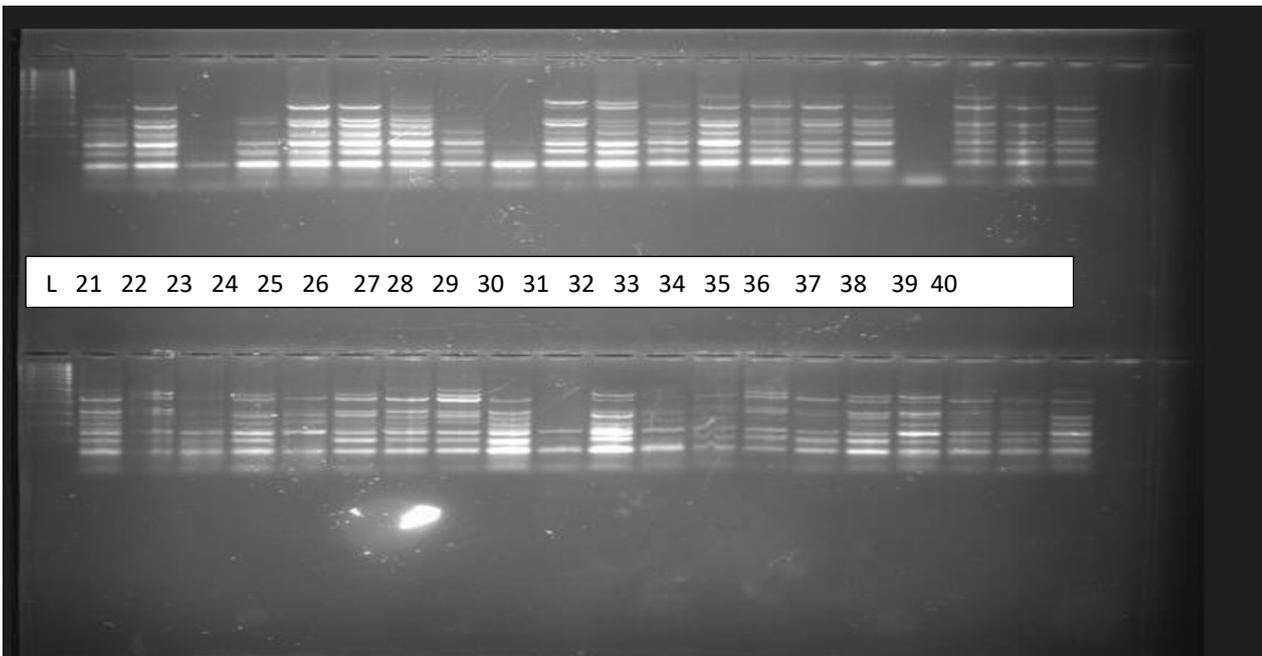


Plate 2: Polymorphic bands showing the amplicons from cultivar 1-40 PCR amplified by M-30 primers

Genetic variability was determined using SSR markers based on amplification of DNA sequence through annealing of random Primers. Ten random SSR markers (Table 1) were used in the PCR, and the PCR products were visualized on 1 % agarose gels (Fig. 1 and 2). Within the collection Of 40 cultivars from NCRI Badeggi, ten random primers amplified a total of 38 alleles With the PIC values ranging from 0.31 (M 14) to 0.82 (M 30), with an average value of 0.51. 6 out of ten primers used had the PIC value higher than 0.5, therefore 60 % of the primers used were sufficiently polymorphic and are suitable for studying the genetic diversity of rice varieties. The M 14 primer had the lowest PIC value (0.31), followed by M 34 (0.33), M26 (0.36 and M 06 (0.44) and are therefore all moderately.

A dendrogram generated grouped the 40 accessions in to two major clusters (Figure 1). The first major cluster had three sub-clusters. The first sub-cluster had 5 rice cultivars (Faro-52, Faro-44, Faro-56, Faro-37, and Faro-54), the second sub-cluster had 6 rice cultivars (Faro-61, Faro-62, Faro-35, Faro-50, Faro-63, and Faro-64), while the third sub-cluster had 10 rice cultivars. The second major cluster also had two sub-clusters, with the first sub-cluster having 13 rice cultivars and the second sub-cluster having 6 rice cultivars (Faro-21, Faro-30, Faro-23, Faro-17, Faro-18, and Faro-22). It was also observed that Faro-66 and Faro-67 were molecularly identical among the Nigerian rice cultivars sampled.

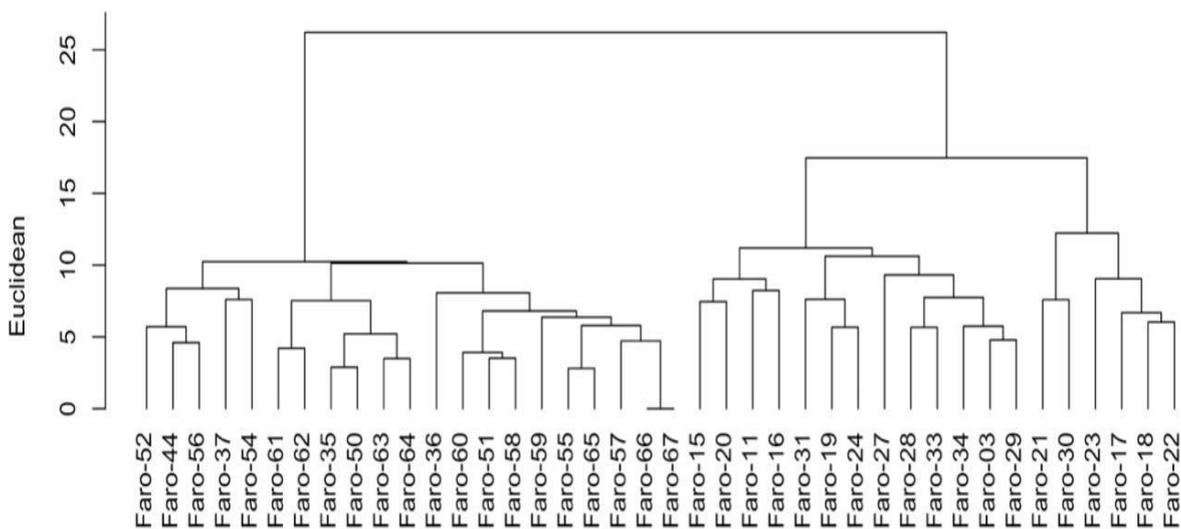


Figure 3. A cluster of the 40 Nigerian rice cultivars based on their molecular data

DISCUSSION

Genetic variation provides breeders with information on the richness and distribution of the available traits in the population, and also informs breeders whether progress can be made in the selection of desirable traits of interest (Sahu *et al.*, 2018). The study revealed a wide range of exploitable variations in trait measurements among the Nigerian rice cultivars evaluated.

SSR markers were assessed on the rice cultivars in the current study. All markers used were amplified in all the rice cultivars evaluated with different levels of polymorphism.

Molecular markers have proven powerful for the assessment of genetic variations (Kiranmayee *et al.*, 2022). Combined with clustering analysis, relationships within and among species have been elucidated. Two major clusters were observed using the molecular data generated from samples. Efiue *et al.* (2014), Suvi *et al.* (2020) and Yang *et al.* (2021) also reported three clusters among the rice genotypes evaluated in their studies using phenotypic characters. Similar to the two clusters generated using molecular data in the study, Thant *et al.* (2021) observed two major clusters among 117 rice genotypes using silicoDART markers. The presence of two major clusters from these studies relative to the current study may indicate there are two population or structure groups within the rice germplasm.

DNA-based markers have proven powerful for the elucidation of genetic diversity/variation and relationships within and among species (Kiranmayee *et al.*, 2022). Hence, genetic diversity indices which include allele number, heterozygosity, polymorphic information content (PIC), and Shannon index to mention a few, provide an estimate of the genetic diversity level within plant germplasms. The study observed 2–7 alleles with an average of 3.8 alleles generated among the markers with a moderate average PIC value. This shows favourable allelic diversity, which is necessary to evaluate genetic diversity (Suvi *et al.*, 2020). This was similar to reports from Pratap *et al.* (2020) who also observed 2–6 alleles from SSR markers used and a PIC value of 0.72, Kiranmayee *et al.* (2022) also reported a PIC value of 0.73 with markers generating 2–8 alleles. The study further revealed a moderate level of diversity among Nigerian rice cultivars evaluated as indicated by the diversity indices used in the study. However,

significantly higher mean allele numbers were reported by Chemutai *et al.* (2016), Rahman *et al.* (2012), and Prathepha (2012) in their studies. Hence, the variations in PIC values may be attributed to marker selection and test genotype variability, as suggested by Suvi *et al.* (2020). Hence, the average PIC of 0.51 in this study indicates a high diversity within the Nigerian rice cultivars.

An average level of heterozygosity was observed in this study. Lower heterozygosity has been reported in the studies of Nachimuthu *et al.* (2015), Mangosongo *et al.* (2020) and Suvi *et al.* (2020). However, the low heterozygosity in these studies has been attributed to the autogamous reproduction nature of rice (Suvi *et al.*, 2020), which increases the degree of inbreeding within the rice population (Ndjiondjop *et al.*, 2018).

CONCLUSION

The 40 Nigerian cultivars used in this study showed high levels of variability in genotypic evaluations, indicating a wide range of alleles in the accessions used for this study. Hence, some of these cultivars with desirable phenotypic traits, such as Faro-44, Faro-59, Faro-60, Faro-61 and Faro-67 can be selected or included in the agronomic development of Nobel Resilient, early maturing and High yielding Nigerian rice, to promote food security and National Economic Development.

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