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## Screening of Rice Genotypes for Adaptability to Ferruginous Ultisol using Morphological, Physiological and Molecular Makers

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### Abstract

Rice production in many tropical regions is constrained by ferruginous ultisol soils characterized by high iron toxicity and poor nutrient availability, which negatively affect plant growth and productivity. Despite the prevalence of ferruginous soils in many rice-growing regions. Limited information exists on the adaptability and growth performance of different rice genotypes under such soil conditions. Therefore, this study evaluated 10 rice genotypes cultivated in ferruginous ultisol soils to identify varieties with superior adaptability based on morphological, physiological and molecular characteristics in Delta and Edo States, Nigeria. The selected genotypes were commonly grown in the region and were obtained from the Delta Agricultural Procurement Agency, Ibusa, Delta State. Rice seeds were grown for 17 weeks. Germination percentage, morphometric and biomass parameters of all the rice genotypes were assessed. Results revealed that FARO 60 consistently outperformed other genotypes in yield-related traits, including leaf number, leaf area and fresh leaf weight in ferruginous ultisol condition. Molecular analysis using PCR revealed that FARO 60 contained both GN1a and GS2 marker genes associated with improved grain yield and plant architecture. These findings underscore the significance of genotype selection for boosting rice productivity in nutrient-deficient soils and establish FARO 60 as a promising genotype for cultivation in ferruginous ultisols of Southern Nigeria. The results have broad implications for rice breeding initiatives focused on sustainability and food security in sub-Saharan Africa.

**Keywords:** Rice genotypes; Ferruginous ultisol; GN1a; GS2; Rice growth and productivity yield; Responsive genes.

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## INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most highly consumed crops globally, with over 3.5 billion people depending on it as their food (FAO, 2020). In Africa, rice production plays a vital role in food security and sustains the livelihoods of millions of farmers, processors and traders (Africa Rice Center, 2019; Ikhajagbe & Musa, 2020). As a starchy grain, rice contributes approximately 23% of global per capita caloric intake (Musa & Ikhajagbe, 2023). In Nigeria, per capita rice consumption is estimated at 150 kg annually (Musa & Ikhajagbe, 2024) with a steadily increasing demand. Despite its importance, rice production in Nigeria faces numerous challenges, including poor soil properties, recurrent drought, and low-yielding varieties (Bado et al., 2015). Notably, soil degradation-characterized by low pH, low soil water holding capacity, low soil phosphorus and nitrogen levels-has led to widespread ferruginous soil conditions that constrain rice cultivation in Nigeria (Bara'u et al., 2024). Ferruginous soils, which are characterized by high iron content, low phosphorus, and nitrogen, are a significant challenge to rice production in many African countries, including Nigeria (Monokrousos & Mourouzidou, 2023). These soils are widespread in the savannas and grasslands of West Africa, covering over 40% of the region's land area (Rossi et al., 2023). In Nigeria, majority of the soils in the southern regions such as Edo and Delta States are ferruginous (Musa & Ikhajagbe, 2022; Ojo, 2019). Ferruginous soils pose a significant threat to rice production due to their poor fertility, low water-holding capacity, and high acidity (Liu et al., 2022). The high iron content in these soils can lead to iron toxicity, which can reduce rice yields by up to 50% (Karim et al., 2020). Additionally, the low phosphorus and nitrogen content in ferruginous soils can limit rice growth and development, making it essential to identify rice genotypes that can thrive in these conditions (Fageria et al., 2011). According to Bara'u et al. (2024), cultivation of high-yielding and stress-resistant seed varieties can help in controlling the effects of soil degradation and improve rice production in Africa. In recent years, the agricultural sector has witnessed unprecedented progress in the rice breeding program, with the development of high-yielding and stress-tolerant varieties (Karim et al., 2020). The use of molecular markers, such as the GN1a and GS2 genes, has shown to be effective in identifying rice genotypes with improved yield and stress tolerance (Zhao et al., 2020; Bara'u et al., 2024). The GN1a gene, which is associated with grain number, is a key regulator of rice yield (Tubehleh et al., 2023), while the GS2 gene, which is involved in grain size, is an important determinant of rice quality (Korsakov et al., 2023). Morphological and physiological traits of rice, such as plant height, tiller number, panicle characteristics, leaf area, percentage germination, total soluble sugar levels among others, remain fundamental in determining the adaptability of rice genotypes to specific environmental conditions or soil types (Musa & Ikhajagbe, 2020a; Musa & Ikhajagbe, 2024). This study aimed at evaluating the growth performance and adaptability of selected rice genotypes cultivated in ferruginous ultisol soil using morphological,

physiological and molecular approaches. The outcome will provide valuable guidance to breeders, farmers, and policymakers in selecting optimal genotypes for challenging soil conditions and contribute to the development of improved rice varieties for sustainable cultivation.

## Materials and methods

### Study area

This study was conducted at the experimental garden of the Department of Biology and Forensic Science, Admiralty University of Nigeria (6.16471 °N, 6.57544 °E), located along Ibusa-Ogwashuiku expressway in Delta State. The experiment was carried out between January and May 2024.

### Preparation of soil used in the experiment

Soils samples previously collected and characterized by Musa & Ikhajagbe (2020b) from six locations across Edo and Delta States were pooled to form composite sample. The soil was placed in experimental pots (30 x 25 cm), arranged in three replicates for each genotype.

### Soil physiochemical parameters:

Composite soil was air-dried at room temperature of 22-25°C for 30 minutes and then analyzed for organic matter concentration in the soil (SOM), available phosphorus, cation exchange capacity (CEC), pH, total nitrogen, organic carbon (OC), available micronutrients electrical conductivity, soil texture class and maximum water holding capacity using the methods of Musa & Ikhajagbe (2020b). Soil iron concentration was determined using a method described by Calicioglu et al. (2019).

### Rice genotypes

Ten rice genotypes commonly cultivated in Delta and Edo State were sourced from the Delta Agricultural Procurement Agency, Ibusa, Delta State. The selected genotypes included FARO44, FARO57, FARO59, FARO60, FARO64 and FARO67, Art, CHAOTA, HHZ-1 and CP 801. The seeds were sorted in the experimental tray (50 x 50 cm) and allowed for aeration prior to sowing.

### Seeds sowing

Seeds of the selected rice genotypes were sown on January 8, 2024, and harvested on May 5, 2024. Seed variability test were carried out according to (AOSA, 2000) guidelines. Seven seeds were planted in each pot. Each pot was replicated three times and were arranged using a completely randomized design. The experiment was conducted in open field condition, in the Biological Garden of Admiralty University of Nigeria with moderate rainfall and relative humidity (< 120-200 cm) during the growing period.

### Germination assessment

Germination percentage was evaluated five days after sowing (DAS), as the most pronounced differences among genotypes occurred at this stage. The germination percentage was calculated using the formula outlined by ISTA (2005):

$$\text{Germination [\%]} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100 \dots (1)$$

**DNA assays**

Fourteen days after sowing, ten seedlings from each genotype were collected from experimental pots for DNA extraction.

**Genomic DNA extraction**

Genomic DNA of the 10 rice genotypes was extracted using a standard Plant/Seed Miniprep Kit according to the manufacturer’s protocol. Beta-mercaptoethanol was added to the lysis buffer to a final dilution of 0.5% (v/v) using 250 µL per 50 mL. Approximately 150 mg of seedling

tissue was transferred into a 2.0 mm lysis tube containing 750 µL Bashing Bead Buffer and processed at maximum speed for 5 minutes, following the method of [Bara’u et al. \(2024\)](#).

**Primers design**

Gene-specific primers targeting the GN1a and GS2 genes regions were designed using VECTOR NTI (Version 11.5 Advanced) ssoftware. The primers were used for conventional PCR amplification to detect the presence of GN1a and GS2 gene fragments in the DNA extracted from the rice genotypes. The PCR products were subsequently separated by agarose gel electrophoresis and visualized under UV illumination

**Table 1:** Primer design for the assayed genes and band sizes

Gene	Forward primer	Reverse primer	Amplicon size (bp)
GN1a	5’-ATGCGTGCTGGTGGTGG-3’	5’-TCACGCTGCTGCTGCTG-3’	185
GS2	5’-ATGGCTGCTGGTGGTGG-3’	5’-TCACGCTGCTGCTGCTG-3’	390

**PCR Amplification of GN1a and GS2**

PCR amplification was performed using gene-specific primers designed to target conserved regions of the GN1a and GS2 genes. The primer sequences were obtained based on published gene sequences and designed using vector NTI as stated above. The PCR reaction mixture consisted of template DNA, forward and reverse primers, PCR master mix, and nuclease-free water as documented in [Bara’u et al. \(2024\)](#), as in [Table 1](#). PCR conditions included an initial denaturation at 94°C for 5 minutes, followed by 30 cycles of 94°C for 30 seconds, annealing at 60°C for 30 seconds (for GN1a and GS2) and extension at 72°C for 1 minutes. A final extension was performed at 72°C for 5 minutes. The PCR products were resolved on 1% agarose gel stained with ethidium-bromide in 1x TAE buffer. A 100 bp DNA ladder (6 µL) was loaded at both gel ends. Electrophoresis was conducted for 35 minutes at 120 V and 100 Ma. Gels were visualized under UV illumination. Bands corresponding to the expected fragment sizes of GN1a and GS2 genes indicated successful amplification of the targeted gene regions. The bands were converted to JPEG format for documentation.

**Morphological parameters**

Fresh shoot length, panicle length, length of the first leaf and length of internodes were measured in centimeters using a transparent ruler at designated intervals. Tiller number was recorded at the time of harvest. The rice crop was harvested when the grains were turning yellowish brown at 118<sup>th</sup> day after sowing. Internode length (cm) and panicle length (cm) were also measured at the time of harvest. The internode was described as the distance from the coleoptile to the first node. Fresh shoot length (cm) and length of the first leaf (cm) were measured at 4-week intervals from the 4<sup>th</sup> week after sowing till the final harvest day.

**Physiological parameters**

To investigate the yield properties of the different rice genotypes planted in the test soil, physiological parameters were studied. Fresh leaves from each genotype were

collected on the final day of harvest for total soluble sugar (TSS) and alpha amylase (AA) analysis. Leaves were dried at 70°C for 24 hours, following method of [Rossi et al., \(2023\)](#). Chlorophyll content index (CCI) was determined using an Apogee chlorophyll meter.

**Biomass parameters of test plants**

Leaf area (cm<sup>2</sup>) was measured using the Leaf-IT mobile application ([Julian et al., 2017](#)) on the final day of harvest. The number of leaves was counted on the final day of harvest. Fresh leaves weight (g) was determined using an analytical balance on the harvest day. Chlorosis and necrosis percentage were calculated using the formula of [Ikhajiagbe & Musa \(2020\)](#) as:

$$\text{LC\&N [\%]} = \frac{\text{Number of affected leaves}}{\text{Total number of leaves}} \times 100 \dots \dots \dots (2)$$

LC&N = Percentage leaf chlorosis and necrosis

$$\text{Medullary Index} = \frac{\text{Medullary Diameter}}{\text{Hair Shaft Diameter}} \dots \dots \dots (3)$$

**Statistical analysis**

All data were expressed as means ± standard errors (SE) from three replicates. Data was subjected to one-way analysis of variance and means were separated using Turkey’s Honesty Significant Difference (HSD) test at *p* < 0.05.

**Results**

**Soil physicochemical properties**

The composite soil showed characteristics typical of ferruginous ultisol. It was acidic (pH 5.01) and rich in iron (210.01 mg/kg), but low in phosphorus (7.12 mg/kg) and nitrogen (0.62%). The soil had a loamy-sandy texture and a relatively low cation exchange capacity (3.70 cmol/kg). Water-holding capacity was also low (62.12%) and organic content of the soil was also low (7.40%). Detailed parameters are presented in [Table 2](#).

**Table 2:** Physical and chemical parameters of the study soil

Parameters	Sample soil
Soil organic matter (%)	7.40 ± 0.56
Soil available phosphorus (mg/kg)	7.12 ± 1.00
CEC (cmol/kg)	3.70 ± 0.02
pH	5.01 ± 0.21
Total nitrogen (%)	0.62 ± 0.01
Total organic carbon (%)	0.41 ± 0.11
Na <sup>+</sup> (meq/100g)	3.09 ± 0.29
Al (meq/100g)	6.23 ± 1.22
Exchangeable acidity (meq/100g)	0.16 ± 0.13
Water-holding capacity (%)	62.12 ± 0.12
Fe (mg/kg)	210.01 ± 2.44
Soil textural class	Loamy-sandy

Fe = Iron, Al = Aluminum, Na = Sodium, CEC= Cation exchange capacity.

**Germination parameter**

Germination percentage differed significantly among genotypes at  $p < 0.05$ . FARO 60 achieved full germination (100%) at day 5 after sowing (DAS0, followed by FARO 44 (92%) and FARO 67 (90%). Delayed germination was observed in FARO 57 and ART (52%). All genotypes exhibited >50% germination at AS (Figure 2).

**Morphological parameters**

Result showed that significant differences ( $p < 0.05$ ) existed among the 10 rice genotypes in terms of their morphology. FARO 60 had the highest fresh shoot length and length of first leaf consistently at all the assayed days (Table 3). Meanwhile, the Art rice genotype showed the lowest fresh shoot length throughout the growing period (Figure 1; Table 3 and 4). Generally, the fresh shoot length and length of first leaf was observed to follow this array FARO 60>FARO 44>FARO 67>CHAOTA> FARO 59>FARO 64>HHZ-1>CP 801>FARO 57>Art. FARO 60 (Table 3) was also showed the highest panicle length (17.6 cm) and has the largest length of internode (cm) compared to the other rice genotypes. However, FARO 60 showed low number of tillers (50), while the CP 801 with low panicle length (5.5 cm) and short internode (5.4 cm) showed the largest tiller number (89).

**Table 3:** Rice shoot length and length of first leaf

Rice genotypes	Fresh shoot length (cm) on				Length of first leaf (cm)			
	4 WAS	8 WAS	12 WAS	16 WAS	4 WAS	8 WAS	12 WAS	16 WAS
FARO 44	34.1 ± 1.5 <sup>b</sup>	48.2 ± 2.5 <sup>b</sup>	62.2 ± 1.1 <sup>b</sup>	76.2 ± 0.3 <sup>b</sup>	10.1 ± 5.7 <sup>b</sup>	21.1 ± 0.3 <sup>b</sup>	27.2 ± 0.2 <sup>b</sup>	35.2 ± 0.3 <sup>b</sup>
FARO 57	25.0 ± 1.7 <sup>e</sup>	29.1 ± 5.7 <sup>h</sup>	32.1 ± 2.1 <sup>f</sup>	43.2 ± 2.2 <sup>h</sup>	07.0 ± 1.4 <sup>f</sup>	08.0 ± 3.1 <sup>g</sup>	14.2 ± 0.1 <sup>h</sup>	21.2 ± 0.3 <sup>h</sup>
FARO 59	28.2 ± 0.3 <sup>d</sup>	41.1 ± 0.5 <sup>e</sup>	53.1 ± 0.4 <sup>d</sup>	60.9 ± 1.7 <sup>e</sup>	11.1 ± 0.4 <sup>c</sup>	16.2 ± 2.1 <sup>e</sup>	21.1 ± 1.0 <sup>e</sup>	29.6 ± 1.2 <sup>d</sup>
Art	19.1 ± 0.4 <sup>f</sup>	29.2 ± 1.2 <sup>h</sup>	32.3 ± 1.1 <sup>f</sup>	39.6 ± 0.4 <sup>i</sup>	07.0 ± 2.1 <sup>f</sup>	08.0 ± 0.3 <sup>g</sup>	11.3 ± 0.9 <sup>i</sup>	18.4 ± 1.2 <sup>i</sup>
CHAOTA	31.1 ± 0.1 <sup>c</sup>	43.1 ± 1.2 <sup>d</sup>	53.3 ± 1.4 <sup>d</sup>	64.6 ± 1.2 <sup>d</sup>	11.1 ± 3.1 <sup>c</sup>	18.1 ± 2.0 <sup>d</sup>	20.1 ± 1.1 <sup>d</sup>	29.7 ± 1.1 <sup>d</sup>
FARO 60	36.3 ± 0.9 <sup>a</sup>	51.4 ± 0.3 <sup>a</sup>	70.7 ± 2.5 <sup>a</sup>	85.7 ± 1.8 <sup>a</sup>	13.2 ± 2.1 <sup>a</sup>	23.2 ± 0.1 <sup>a</sup>	29.3 ± 1.1 <sup>a</sup>	40.3 ± 1.9 <sup>a</sup>
HHZ-1	25.3 ± 2.1 <sup>e</sup>	31.2 ± 1.5 <sup>g</sup>	46.4 ± 0.1 <sup>e</sup>	58.8 ± 0.1 <sup>f</sup>	08.4 ± 0.7 <sup>d</sup>	10.1 ± 0.1 <sup>g</sup>	17.2 ± 0.5 <sup>f</sup>	25.2 ± 0.3 <sup>f</sup>
CP 801	25.2 ± 3.0 <sup>e</sup>	31.1 ± 2.5 <sup>g</sup>	46.4 ± 0.4 <sup>e</sup>	52.9 ± 1.2 <sup>g</sup>	09.2 ± 2.5 <sup>e</sup>	10.2 ± 3.1 <sup>g</sup>	16.3 ± 0.3 <sup>g</sup>	23.1 ± 0.5 <sup>g</sup>
FARO 64	28.1 ± 3.0 <sup>d</sup>	40.2 ± 1.0 <sup>f</sup>	53.3 ± 2.1 <sup>d</sup>	58.5 ± 2.5 <sup>f</sup>	10.1 ± 2.1 <sup>b</sup>	15.2 ± 0.8 <sup>f</sup>	20.7 ± 0.2 <sup>d</sup>	27.1 ± 0.1 <sup>e</sup>
FARO 67	34.1 ± 1.1 <sup>b</sup>	46.1 ± 1.0 <sup>c</sup>	59.3 ± 1.7 <sup>c</sup>	68.5 ± 1.2 <sup>c</sup>	10.1 ± 0.5 <sup>b</sup>	20.1 ± 0.9 <sup>c</sup>	23.4 ± 0.3 <sup>c</sup>	32.2 ± 0.1 <sup>c</sup>

Values are expressed as Mean ± SE of three replicates. Means followed by the same superscript letters within a column are not significantly different according to HSD test at ( $p > 0.05$ ). WAS= Weeks after sowing

**Table 4:** Panicle length, length of internodes and tiller number

Rice genotypes	Panicle Length (cm)	Length of the Largest Internode (cm)	Tiller Number
FARO 44	12.1 ± 0.2 <sup>b</sup>	09.3 ± 0.8 <sup>a</sup>	76 ± 0.9 <sup>e</sup>
FARO 57	05.2 ± 0.4 <sup>e</sup>	09.1 ± 0.9 <sup>c</sup>	50 ± 0.3 <sup>g</sup>
FARO 59	09.1 ± 0.3 <sup>d</sup>	10.1 ± 0.9 <sup>a</sup>	67 ± 0.4 <sup>e</sup>
Art	05.4 ± 0.1 <sup>e</sup>	04.3 ± 2.1 <sup>f</sup>	85 ± 0.4 <sup>b</sup>
CHAOTA	10.1 ± 0.1 <sup>c</sup>	04.9 ± 0.1 <sup>f</sup>	77 ± 0.1 <sup>c</sup>
FARO 60	17.6 ± 0.2 <sup>a</sup>	08.3 ± 1.3 <sup>d</sup>	50 ± 1.0 <sup>g</sup>
HHZ-1	09.7 ± 0.1 <sup>d</sup>	05.9 ± 1.0 <sup>e</sup>	60 ± 1.2 <sup>f</sup>
CP 801	05.5 ± 0.1 <sup>e</sup>	05.4 ± 1.0 <sup>e</sup>	89 ± 1.1 <sup>a</sup>
FARO 64	09.6 ± 0.8 <sup>d</sup>	05.4 ± 1.0 <sup>e</sup>	85 ± 0.8 <sup>b</sup>
FARO 67	12.5 ± 0.9 <sup>b</sup>	09.3 ± 1.2 <sup>b</sup>	70 ± 0.6 <sup>d</sup>

Values are expressed as Mean ± SE of three replicates. Results with similar alphabets as superscripts on the same column did not differ from each other ( $p > 0.05$ ).



Figure 1: Rice morphological parameters at 3WAS

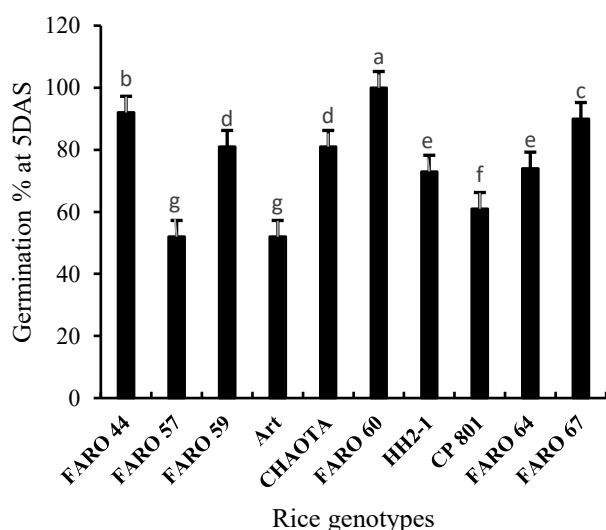


Figure 2: Rice seed percentage germination at 5 days after sowing. DAS= Days after sowing

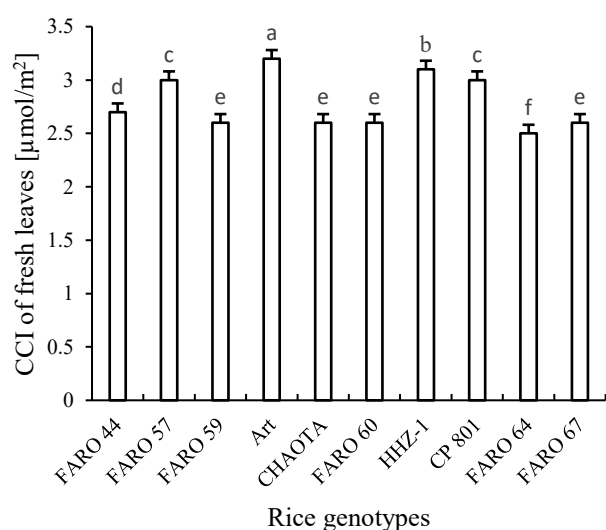


Figure 3: Chlorophyll content index of rice genotypes at harvest day

**Physiological parameters**

Significant differences ( $p < 0.05$ ) in the TSS and AA levels of the fresh rice leaves at the harvest day were observed (Table 5). The FARO 60 rice genotypes showed the highest TSS (66 mg/g<sup>-1</sup>FW) compared to the other rice genotypes, while the Art rice genotypes showed the lowest (47 mg/g<sup>-1</sup>FW). However, the Art rice genotype showed significantly ( $p < 0.05$ ) higher AA content (35U) compared to the FARO 60 rice genotype (28U). Significant differences were also observed in the AA levels among all the assayed rice genotypes. The chlorophyll content index (CCI) was observed to be higher in the Art genotype (3.2 µmol/m<sup>2</sup>), while the lowest (2.6 µmol/m<sup>2</sup>) was observed in the FARO 59, CHAOTA, FARO 60 and FARO 67 rice genotypes (Figure 3).

Table 5: Physiological parameters of rice genotypes

Rice genotypes	TSS [mg/g <sup>-1</sup> FW]	AA [U]
FARO 44	60 ± 0.1 <sup>b</sup>	22 ± 0.4 <sup>g</sup>
FARO 57	47 ± 0.3 <sup>c</sup>	34 ± 0.3 <sup>b</sup>
FARO 59	58 ± 0.5 <sup>c</sup>	28 ± 0.4 <sup>e</sup>
Art	47 ± 0.3 <sup>f</sup>	35 ± 0.1 <sup>a</sup>
CHAOTA	60 ± 0.7 <sup>b</sup>	26 ± 0.3 <sup>f</sup>
FARO 60	66 ± 0.5 <sup>a</sup>	28 ± 1.5 <sup>e</sup>
HHZ-1	51 ± 0.6 <sup>c</sup>	32 ± 0.8 <sup>c</sup>
CP 801	51 ± 0.3 <sup>c</sup>	30 ± 0.4 <sup>d</sup>
FARO 64	56 ± 0.3 <sup>d</sup>	34 ± 0.3 <sup>b</sup>
FARO 67	60 ± 0.7 <sup>b</sup>	28 ± 0.5 <sup>e</sup>

Values are expressed as Mean ± SE of three replicates. Results with similar alphabets as superscripts on the same column did not differ from each other ( $p > 0.05$ ).

**Biomass parameters**

FARO 60 genotype demonstrated superior vegetative biomass metrics. For example, the FARO 60 genotypes showed the highest number of leaves (60) at harvest day, with the highest leaf area (4.4 cm<sup>2</sup>) compared to the other rice genotypes. Furthermore, the FARO 60 was also observed to have the highest weight of fresh leaves (5.2 g<sup>-1</sup>) at harvest day, which was found to be higher than all the other rice genotypes. About 83.3 % of the leaves produced by the CP 801 rice genotype was observed to show signs of chlorosis and necrosis. While only 15% of the leaves produced by the FARO 60 rice genotype showed signs of chlorosis and necrosis (Table 6).

**Gene amplification**

PCR amplification using gene-specific primers produced fragments of the expected sizes for (GNIa and GS2) in the selected rice genotypes (Figure 4). FARO 60 (1), FARO 44 (7), FARO 67 (8), CHAOTA (9) and FARO 59 (10) showed clear double bands (185 bp and 390 bp), indicating the presence of both genes. FARO 64 (2), HHZ-1 (3), CP 801

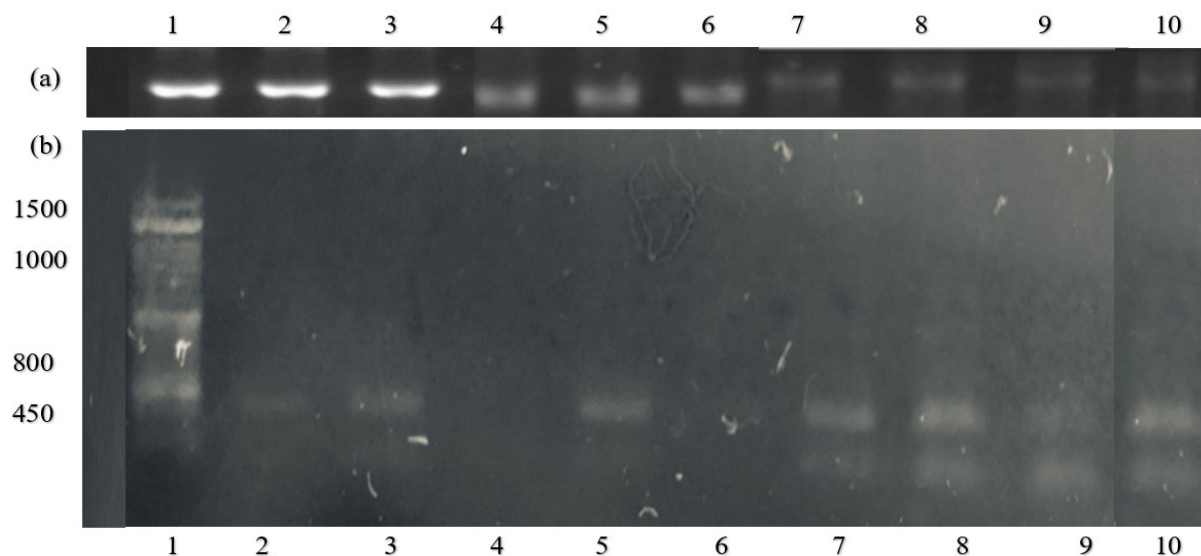
(5) exhibited only the GN1a band. FARO 57 and ART displayed faint single bands, suggesting weak or partial amplification, potentially due to PCR inhibitors or contamination. Selected amplicons were sequenced, and

BLAST analysis against the NCBI GeneBank database confirmed 99-100% identity with reference GN1a and GS2 sequences, validating the presence of these genes.

**Table 6:** Biomass parameters of rice genotypes

Rice genotypes	Area of the Largest Leaf (cm <sup>2</sup> )	Number of leaves	Number of Leaves with Signs of Necrosis and Chlorosis	Weight of Fresh Leaf (g <sup>-1</sup> )
FARO 44	4.2 ± 0.2 <sup>c</sup>	52 ± 0.4 <sup>b</sup>	14 ± 0.1 <sup>g</sup>	4.6 ± 0.1 <sup>b</sup>
FARO 57	3.2 ± 0.2 <sup>f</sup>	22 ± 0.3 <sup>f</sup>	17 ± 0.1 <sup>f</sup>	2.7 ± 0.1 <sup>e</sup>
FARO 59	4.2 ± 0.1 <sup>c</sup>	42 ± 0.4 <sup>d</sup>	21 ± 0.1 <sup>c</sup>	3.2 ± 0.1 <sup>c</sup>
Art	3.2 ± 0.2 <sup>f</sup>	20 ± 0.1 <sup>g</sup>	19 ± 0.1 <sup>e</sup>	2.7 ± 0.1 <sup>e</sup>
CHAOTA	4.1 ± 0.5 <sup>d</sup>	50 ± 0.3 <sup>c</sup>	20 ± 0.1 <sup>d</sup>	3.1 ± 0.1 <sup>d</sup>
FARO 60	4.4 ± 0.3 <sup>a</sup>	60 ± 1.5 <sup>a</sup>	09 ± 0.1 <sup>i</sup>	5.2 ± 0.1 <sup>a</sup>
HHZ-1	4.0 ± 0.4 <sup>e</sup>	36 ± 0.8 <sup>e</sup>	28 ± 0.1 <sup>b</sup>	3.1 ± 0.1 <sup>d</sup>
CP 801	4.0 ± 0.3 <sup>e</sup>	36 ± 0.4 <sup>e</sup>	30 ± 0.1 <sup>a</sup>	3.2 ± 0.1 <sup>c</sup>
FARO 64	4.3 ± 0.1 <sup>b</sup>	36 ± 0.3 <sup>e</sup>	21 ± 0.1 <sup>c</sup>	3.1 ± 0.1 <sup>b</sup>
FARO 67	4.2 ± 0.2 <sup>c</sup>	50 ± 0.5 <sup>c</sup>	12 ± 0.1 <sup>h</sup>	4.6 ± 0.1 <sup>b</sup>

Values are expressed as Mean ± SE of three replicates. Results with similar alphabets as superscripts on the same column did not differ from each other (*p* > 0.05).



**Figure 4:** PCR bands of 10 rice genotypes. FARO 60=1, FARO 44=2, FARO 67=3, CHAO =4, FARO 59 =5, FARO 64 =6, HHZ-1 =7, CP 801 =8, FARO 57 =9, ART =10.

## Discussion

The physicochemical profile of the composite soil characterized by high iron concentration, acidity, low bioavailable phosphorus and limited water-holding capacity confirms its classification as ferruginous ultisol. This aligns with previous findings by Musa & Ikhajiagbe (2020b), who defined ferruginous ultisols as low-fertility, iron-rich soils with poor agronomic potential. These conditions are known to inhibit plant growth and development, particularly in sensitive crops like rice (Tubeileh et al., 2023). The high germination percentage observed in the FARO 60 rice genotype under the ferruginous ultisol is consistent with the work of Kumar et al. (2019) who observed that certain rice genotypes may have more efficient iron uptake and

utilization mechanism compared to other genotypes. This property may make those rice genotypes to thrive in soils with poor growing conditions such as high acidity and iron levels (Yang et al., 2018). The exceptional performance of the FARO 60 rice genotype in terms of morphology on ferruginous ultisol suggests its superior ability to adapt to the challenging conditions of ferruginous ultisol. These findings are in agreement with a previous study by Singh et al., (2017) who reported the importance of shoot length and leaf growth in determining rice yield and productivity. In fact, shoot length has been identified as a key trait for rice breeding programs aimed at improving yield and stress tolerance (TenHuf et al., 2023). The low tiller number observed in FARO 60, while CP 801 showed the largest tiller number

suggests that tiller number may not be a reliable indicator of rice yield and productivity on ferruginous ultisol, since different plants have different growth habit and architecture that may allow it to allocate more resources to other plants parts rather than tiller production. Previous studies have reported that tiller number can be negatively correlated with grain yield in some rice genotypes (Khush, 2015). The high TSS observed in the FARO 60 rice genotype at harvest indicated superior ability of the FARO 60 to accumulate carbohydrates under ferruginous stressed conditions. This observation is in agreement with previous studies that have reported the importance of TSS content in determining rice yield and productivity (Singh et al., 2017; Monokrousos & Mourouzidou, 2023). In contrast, the low TSS content observed in the ART rice genotype may suggest its poor adaptability to ferruginous ultisol. However, the high alpha amylase content (an enzyme involved in starch breakdown) which was observed in the ART rice genotype may indicate that the rice genotype has a different carbohydrate metabolism pathway. This is because the same ART rice genotype was observed showing higher chlorophyll content index suggesting a more efficient photosynthetic pathway. The differences in TSS content, AA levels and CCI among the rice genotypes suggest that they have different strategies for coping with the ferruginous stress. FARO 60, appeared to maintain letter vegetative growth under ferruginous soil conditions. In contrast, ART rice genotype has shown to be better able to rapidly mobilize starch reserves and capture light energy. The high incidence of chlorosis and necrosis in CP801 (83%) and ART (95%) may suggest that these genotypes are more susceptible to iron toxicity and nutrient deficiencies associated with ferruginous ultisol. In contrast, the significantly low incidence of leaf damage (15%) observed in FARO 60 may suggest its tolerance to the stressful soil conditions. This result aligns with a previous study by Wang et al. (2015) who reported that rice genotypes with enhanced tolerance to iron toxicity tend to have lower percentage of leaf necrosis and chlorosis and looks healthy. In general, the superior performance of the FARO 60 rice genotype compared to the other genotypes in ferruginous ultisol can be linked to the genetic make, which may bring about improved adaptability to stressful soil conditions. Previous research identified several quantitative trait loci (QTLs) that are linked with iron toxicity tolerance in rice (Deng et al., 2013). The presence of clear and double bands for GN1a and GS2 in FARO 60, FARO 44, FARO 67, CHAOTA and FARO 59 suggests that the amplification confirmed the presence of GN1a and GS2 genes in several genotypes. However, the presence of these genes alone does not necessarily determine grain yield performance in rice is often influenced by specific allelic variants and gene expression levels (Fan et al., 2016). The FARO 64, HHZ-1 and CP 801 with a single clear band may indicate the presence of only one of the genes which may have been the reason for the compromise in the vegetative properties of these genotypes. The rice genotypes FARO 57 and ART with single and unclear band may indicate

the presence of inhibitors or contaminants in the PCR reaction.

## Conclusion

This study evaluated the performance of ten rice genotypes in ferruginous ultisol of Delta and Edo States of Nigeria. Ferruginous soil is a challenging soil type characterized by high iron content, low macronutrient levels (N, P, K), low water-holding capacity and acidity. Among the evaluated genotypes, FARO 60 consistently outperformed others (FARO 44, FARO 57, FARO 59, ART, CHAOTA, HHZ-1, CP 801, FARO 64 and FARO 67) across germination, morphological, physiological and biomass parameters. It exhibited the highest leaf number, leaf area, and fresh leaf weight, alongside a markedly lower incidence of foliar chlorosis and necrosis, indicating tolerance to iron toxicity and nutrient stress. This result was confirmed by molecular analysis using PCR which revealed that FARO 60 along with few other rice genotypes possessed both GN1a and GS2 genes, which are associated with improved grain yield and plant architecture. PCR amplification confirmed the presence of GN1a and GS2 genes in several genotypes including FARO 60. However, further studies involving allelic characterization and gene expression analysis are required to determine their direct contribution to yield performance. These findings suggest that FARO 60 has potential as a suitable rice genotype for cultivation in ferruginous ultisol, providing a viable option for farmers to improve rice productivity if they found their selves in ferruginous condition. This study contributes to the understanding of rice genotype performance on ferruginous ultisol and provides valuable insights for breeding programs aimed at developing iron toxicity-tolerant rice varieties. Further research is needed to elucidate the genetic basis of FARO 60's tolerance mechanisms and to explore its potential for cultivation in other challenging soil environments.

### List of abbreviations

SOM = Soil organic matter  
 CEC = Cation exchange capacity  
 OC = Organic carbon  
 Na = Sodium  
 Al = Aluminum  
 AOSA = American official seed analyst  
 CRD = Completely randomized design  
 ISTA = International seed testing association  
 DNA = Deoxyribonucleic acid  
 PCR = Polymerase chain reaction  
 AA = Alpha amylase  
 TSS = Total soluble sugar  
 CCI = Chlorophyll content index  
 DAS = Days after sowing  
 WAS = Weeks after sowing

### Declarations

Ethics approval and consent to participate

Not applicable

### Consent for publication

Not applicable

### Availability of data and materials

All data used in this research were generated as primary data by the researchers. The raw data are with the researchers and can be obtained once the researchers are approached

#### Declaration of Competing Interest

The authors have studied the final version of the manuscript and unanimously agreed to submit it for publication consideration in *South African Journal of Botany*.

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#### Author's contributions

Musa Saheed designed the study and carried out the research under the supervision of Beckley Ikhajiagbe. Both authors contributed to data analysis and interpretation. Musa Saheed prepared the first draft, while Beckley Ikhajiagbe edited the final draft of the manuscript. The authors read and approved the final manuscript.

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## References

- Africa Rice Center (AfricaRice). (2019). Annual Report: Toward rice-based food systems transformation in Africa. Abidjan, Côte d'Ivoire: 28 pp.
- Bado, S., Bazongo, P., Son, G., Kyaw, M., Forster, B., Nielen, S., Lykke, A., Ouedraogo, A., Bassole, I. (2015). Physicochemical characteristics and composition of three morphotypes of *Cyperus esculentus* tubers and tuber oils. *Journal of Annual Methods in Chemistry*. 6(7): 35-47.
- Bara'u, Y., Salaudeen, A., Gana, S., Anas, A., Mohammed, A., Makarfi, A. (2024). Evaluation of yield indices and identification of yield responsive genes (GN1a and GS3) of selected rice genotypes. *Asian Journal of Biological Sciences*. 17(4): 123-129.
- Dang, F., Wang, L., Zhang, X. (2013). Quantitative trait loci for iron toxicity tolerance in rice. *Euphytica*. 191(12): 251-262.
- Calicioglu, O., Flammini, A., Bracco, S., Bellu, L., Sims, R. (2019). The future challenges of food and crops: trends and solutions. *Sustainability*. 11: 22-29.
- Fageria, N., dos-Santos, A., Coelho, A. (2011). Growth, yield and yield components of lowland rice as influenced by ammonium sulfate and urea fertilization. *Journal of Plant Nutrition*. 4: 371-386.
- Fan, C., Xing, Y., Mao, H. Lu, T., Han, B. Xu, C. & Zhang, Q. (2016). GS2, a major QTL for grain length and weight, encodes a putative transmembrane protein. *Theoretical and Applied Genetics*. 112(6): 1164-1171.
- FAO (2020). FAOSTAT. FAO. Food and Agriculture Organization of the United Nations, Rome, Italy.
- Ikhajiagbe, B. & Musa, S. (2020a). Application of biosynthesized nanoparticles in the enhancement of growth and yield performances of rice (*Oryza sativa* var. Nerica) under salinity conditions in a ferruginous ultisol. *FUDMA Journal of Science*. 4(1): 120-132.
- Ikhajiagbe, B. & Musa, S.I. (2020b). The effects of salicylic acid on the germination and early seedling growth of pigeon pea (*Cajanus cajan*). *Notulae Sci. Biol.* 12(3): 683-692.
- Julian, S., Giso, P. & Holger, K. (2017). Leaf-IT: An android application for measuring leaf area. *Ecology and Evolution*. 7: 9731-9738.
- Karim, K., Ifie, B., Dzidzienyo, D., Danquah, A. & Blay, C. (2020). Genetic characterization of cassava (*Manihot esculenta* Crantz) genotypes using agro-morphological and single nucleotide polymorphism markers. *Physiol. Mol. Biol. Plants*. 26: 317-330.
- Khush, G. (2015). What it will take to feed 5.0 billion rice consumers in 2030. *Plant Molecular Biology*. 59(1): 1-6.
- Korsakov, K., Stepanov, A., Pozdnyakov, L. & Yakimenko, O. (2023). Humate-coated urea as a tool for change soil properties. *Agronomy*. 13(2): 19-58.
- Kumar, J., Singh, G. & Sharma, T. (2019). Screening of rice genotypes for iron toxicity tolerance. *International Journal of Plant Physiology*. 24(2): 123-132.
- Liu, M., Song, F., Yin, Z., Chen, P., Zhang, Z., Qi, Z., Wang, B., Zheng, E. (2022). Effects of reduced nitrogen in Maize Fields in Mollisols in Northeast China: A 15N tracing study. *Agronomy*. 12(2): 30-43.
- Monokrousos, N. & Mourouzidou, S. (2023). Ensuring plant nutrition through sustainable soil. Research Progress and Challenges. *Agronomy*. 13(1): 2061-2073.
- Musa, S. & Ikhajiagbe, I. (2024). Evaluation of previously isolated and characterized phosphate-solubilizing bacteria with plant growth-promoting potentials for rice grown in ferruginous ultisol Soil in Benin City, Edo State, Nigeria. *Journal of Applied Science and Environmental Management*. Vol. 28 (12B Supplementary) 4393-4407 Dec. 2024.

<https://dx.doi.org/10.4314/br.v24i1.18>

Musa, S.I. & Ikhajiagbe B. (2020a). Screening bacterial isolates for phosphate Solubilizing capability in a ferruginous ultisol in Benin City, Edo State Nigeria. *Bayero Journal of Pure Applied Science*. 13(2): 14–20.

Musa, S.I. & Ikhajiagbe, B. (2020b). Assessment of physicochemical properties of ferruginous ultisol in Benin City, Edo State – possible impact on plant distribution. *Studia Univer. “Vasile Goldiş”, Ser. Ştiinţele Vieţii*. 30(2): 88–95.

Musa, S.I. & Ikhajiagbe, B. (2023). Seed bio-priming with phosphate-solubilizing bacteria strains to improve rice (*Oryza sativa* L. var. FARO 44) growth under ferruginous ultisol conditions. *Journal of Biotechnology, Computational Biology and Biotechnology*. 104(1): 33-51.

Musa, S.I. & Ikhajiagbe, B. (2022). Rhizo-inoculation of phosphate solubilizing bacteria strains to improve rice (*Oryza sativa* L. var. FARO 44) growth under ferruginous ultisol conditions. *Research Square*. 10(3): 39-42.

Ojo, D., Oduwaye, O. & Ayo-Vaughan, M. (2019). Genetic variability and heritability of yield and yield components in rice (*Oryza sativa* L.). *Nigerian Journal of Genetics*. 25(1): 1-8.

Rossi, G., Beni, C., Benedetti, A., Felici, B. & Neri, U. (2023). Effect of mineral or OFMSW digestate food on leaching. *Agronomy*. 13(1): 1958-1967.

Singh, R., Singh, Y. & Singh, G. (2017). Rice breeding for improved yield and stress tolerance. *Journal of Crop Science and Biotechnology*. 20(2): 257-265.

TenHuf, M., Reinsch, T., Zutz, M., Essich, C., Ruser, R., Buchen-Tschiskale, C. & da Silva, J. (2023). Application techniques on ammonia emission and winter wheat yield. *Agronomy*. 12(2): 34-44.

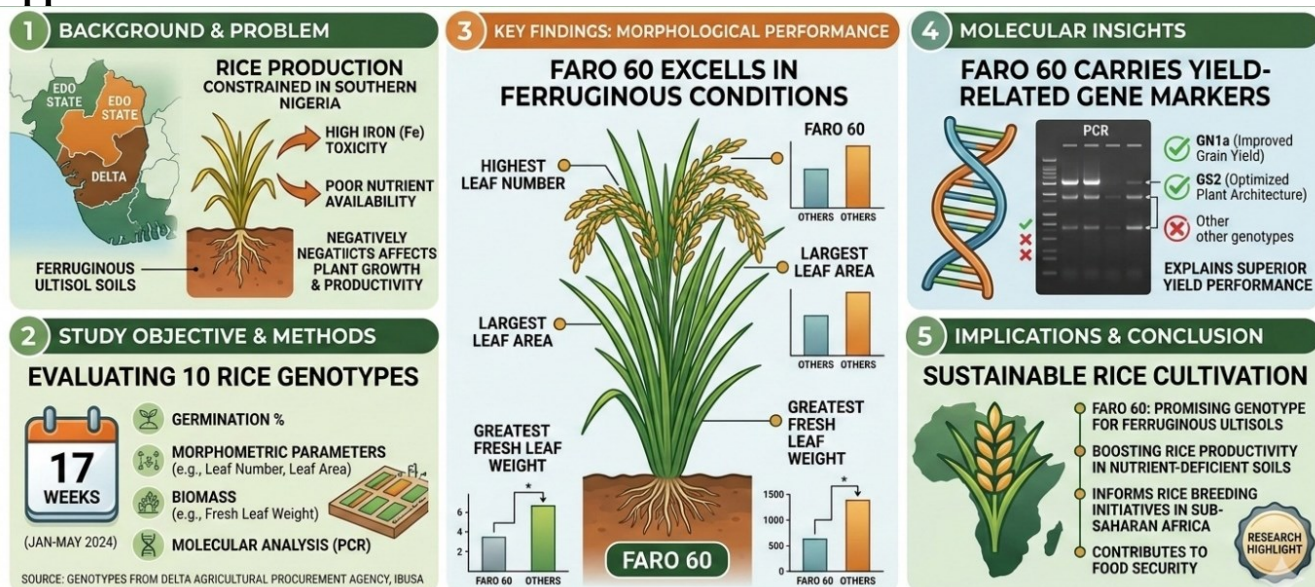
Tubeileh, A., Alam-Eldein, S. & Banks, S. (2023). Effects of polymer-coated urea/urea blends on season conditions in Eastern Canada. *Agronomy*. 13(4): 695-712.

Wang, X., Deng, F. & Zhang, X. (2015). Physiological and molecular mechanisms of iron toxicity tolerance in rice. *Plant and Soil*. 397:(1-13).

Yang, A., Kumar, J. & Li, C. (2018). Iron uptake and utilization in rice: A review. *Journal of Experimental Botany*. 69(11): 2675-2685.

Zhao, M., Lin, Y. & Chen, H. (2020). Improving nutritional quality of rice for human health. *Journal of Plant Biology*. 12(2): 12-34.

## Appendix



Infographic abstract