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Full Length Research Article

Effect of Flood Stress on Growth of Selected Local Rice Genotypes

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ABSTRACT
Flooding poses a severe threat to global rice production, with Nigeria's crop yields being particularly vulnerable. This study investigated the impact of flood stress on three local Nigerian rice genotypes—Kwandala, Yar-gafan, and Yar-dass—by evaluating their morphological, biochemical, and genetic responses. Using controlled submergence experiments, morphological traits were measured across vegetative and booting stages. Biochemical assays quantified key antioxidants (ascorbate, glutathione, tocopherol) and oxidative stress markers (malondialdehyde), while genetic screening and phylogenetic analysis were performed to detect the presence of submergence-tolerance genes SK1 and SK2. Results showed that the genotype of Kwandala exhibited the highest plant height and most favorable antioxidant profile under stress, alongside possessing both SK1 and SK2 genes. In contrast, the other genotypes displayed greater oxidative damage and lacked one or both genes. Overall, Kwandala demonstrated the strongest flood tolerance, attributable to its combined morphological resilience, adaptive antioxidant capacity, and key submergence-tolerance genes. These findings recommend Kwandala as a promising candidate for breeding programs aimed at enhancing flood resilience in Nigerian rice cultivation.
KEYWORDS: Rice, Flood, Abiotic stress, Snorkel genes

INTRODUCTION

Flooding refers to a situation where an area becomes submerged or inundated with water, typically due to heavy rainfall, snowmelt, or overflow of water bodies such as rivers, lakes, or oceans. Flooding can occur suddenly and can cause significant damage to infrastructure, crops, and homes, as well as pose a threat to human and animal life. It is a natural phenomenon that is often exacerbated by human activities such as urbanization and deforestation. In addition to its destructive potential, flooding can also provide benefits such as replenishing water sources and providing nutrients to soil (Das *et al*., 2025). The presence of excess water in soil, which can occur due to waterlogging or submergence, collectively referred to as flooding, can cause abiotic stress and impact the species composition and productivity of various plant communities across the globe. In both natural and man-made wetlands, the vegetation present can be influenced by hydrological patterns, which are determined by the ecophysiological responses of plant species to flooding (Voesenek *et al*., 2004).

Nigeria is one of the leading African countries in rice production, with over 5 million tonnes a year. According to Anugwara and Emakpe (2013), the 2012 floods damaged over 1.9 million hectares of land across the country and reduced food production along floodplains. Rice production in the affected areas was reduced by 22.4%, maize production was reduced by 14.6%, and soybean, cassava, and cowpea production were reduced by 11.2%, 9.3% and 6.3%, respectively. Hattori *et al*. (2009) used positional cloning and a transgenic approach to identify two genes, SK1 and SK2, that regulate the deep-water response in rice. These genes were found to be absent in non-deep-water rice. The researchers performed phylogenetic analysis using the AP2/ERF domain and discovered that the SK genes belong to the ERF subfamily, which is known to be involved in various cellular processes, including hormonal signal transduction (Ohme-Takagi and Shinshi, 1995) and response to biotic and abiotic stresses (Guan *et al*., 2000; Dubouzet *et al*., 2003). The aim is to determine the effect of flood stress on the growth of selected local rice genotypes.

MATERIALS AND METHODS:

Chemicals and Reagents;

Oxalic acid, metaphosphoric acid, trichloroacetic acid (TCA), thiobarbituric acid (TBA), n-butanol, ethanol (absolute and dilute), DTNB Reagent, distilled water, xylene, batophenanthrolein, ferric chloride, phosphoric acid. Phosphotungstate Reagent (PR): sodium tungstate (molybdenum-free), sodium hydrogen phosphate, 3.7 M sulphuric acid, deionised water.

Materials

The materials used in this research include a micropipette, DNeasy Plant Mini kit (QIAGEN), PCR machine, and Alpha imager 2200. Erlenmeyer flasks, beakers, pipettes, test tubes (centrifugal and glass), funnels, filter paper, shaker, centrifuge, water-bath, reflux condenser, magnetic stirrer, spectrophotometer, micropipette, and Pasteur pipette.

Soft Tools

SPSS, CLC Sequence Viewer, Clustal W Omega, NCBI.

Methods

Morphological and Submergence studies were conducted according to the Standard Evaluation System of rice (IRRI, 2002)

Biochemical Test

Lipid peroxidation was determined using the thiobarbituric acid (TBA) test in accordance with Ohkawa *et. al.* (1979). Ascorbate content was determined using the method highlighted by Rutkowski and Grzegorczyk, (1998). α-tocopherol content was determined using the method highlighted by Rutkowski and Grzegorczyk, (1998). Total

reduced glutathione content in the samples was estimated by the method of Boyne and Ellman (1972).

DNA Extraction, PCR and Gel Electrophoresis

DNA was extracted according to the prescribed protocol of the DNeasy Plant Mini Kit (QIAGEN). Samples were run on 1% agarose gel to confirm the presence of the DNA. Two SNP markers were used to amplify the isolated genomic DNA from the respective rice genotypes by PCR, and gel electrophoresis was used to visualize the size polymorphisms of the PCR product.

Gene Sequencing

The Sanger DNA sequencing method was employed in this study to determine the nucleotide sequences of the SK1 and SK2 gene fragments amplified from the local rice genotypes. This method, based on chain-termination with dideoxynucleotides, was chosen for its accuracy and reliability in reading relatively short PCR products (typically up to ~1000 bp). The resulting sequences were then used for phylogenetic analysis, allowing for the comparison of genetic variation and evolutionary relationships between the local genotypes and reference accessions in public databases.

Phylogenetic Analysis

The sequences of the respective genotypes were aligned, and the evolutionary genetic relationship between the similar rice genotypes was constructed using CLC Sequence Viewer v7.7 for Windows.

Statistical Analysis

The data obtained were subjected to analysis of variance (ANOVA) using the statistical package SPSS Version 20. Data were expressed as mean ± S.D. at $p < 0.05$ level of significance. Pearson correlation regression models were used to study associations between different variables. $p < 0.05$ was considered significant.

RESULTS

Plant Height

The plant height difference across three rice genotypes treated between two categories is presented in Figure 1. Plant height among rice genotypes (Kwandala, Yar-gafan and Yar-dass) at vegetative stages ranges between 13.03±1.65 cm and 14.10±0.8 cm, with Yar-gafan having the lowest plant height and Kwandala having the highest plant height. However, there was no significant difference among the genotypes treated with respect to the plant height. On the other hand, among the three rice genotypes in the booting stage, the plant height ranges between 73.67±2.08 cm to 98.33±4.2 cm, with Yar-gafan lowest plant height and Kwandala having the highest plant height. There is a significant increase in plant height in Kwandala as compared with other genotypes ($P < 0.05$).

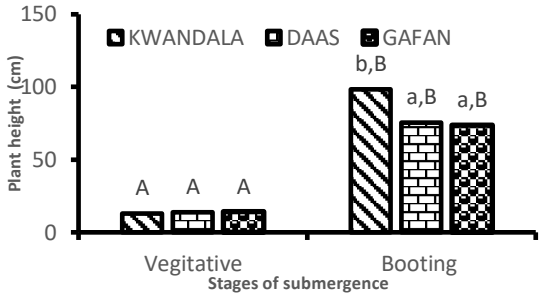

Figure 1: Effect of flood stress on plant height in three rice genotypes.

Values are presented in mean ± standard deviation of three independent determinations. Values in the same group followed by different lowercase letters are significantly different ($P<0.05$). Values in the different categories followed by different uppercase letters are significantly different ($P<0.05$).

Number of Tiller

The number of tiller difference across three rice genotypes treated between two categories is presented in Figure 2. Number of tiller among rice genotypes (Kwandala, Yar-gafan and Yar-dass) at vegetative stages ranges between 5±0.00 and 5.6±1.15, with Kwandala having the lowest number of tiller and Yar-gafan having the highest number of tiller. Among the three rice genotypes in the booting stage, number of tiller ranges between 5.0±0 and 5.6±0.67 with Kwandala having the lowest number of tiller and Yar-gafan having the highest number of tillers. There is no significant difference in the number of tillers across the three rice genotypes in vegetative and booting stages ($P > 0.05$).

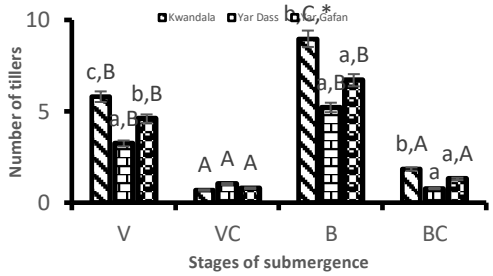

Figure 2: Effect of flood stress on number of tillers in three rice genotypes

Values are presented in mean ± standard deviation of three independent determinations. Values in the same group followed by different lowercase letters are significantly different ($P<0.05$). Values in the different categories followed by different uppercase letters are significantly different ($P<0.05$). VC: Vegetative control, V: Vegetative, B: Booting, BC: Booting control.

Number of Leaves

The number of leaves difference across three rice genotypes treated between the two categories is presented in Figure 3. The number of leaves among rice genotypes (Kwandala, Gafan, and Dass) at vegetative stages ranges between 19.3±3.79 cm and 25.66±4.50 cm, with Kwandala having the lowest number of leaves and Yar-gafan having the highest number of leaves.

Among the three rice genotypes in the booting stage, the number of leaves ranges between 19.00±1.0 cm and 21.66±1.52 cm, with Yar-dass having the lowest number of leaves and Kwandala having the highest number of leaves. However, there is no significant difference in the stages with regard to the number of leaves (P > 0.05).

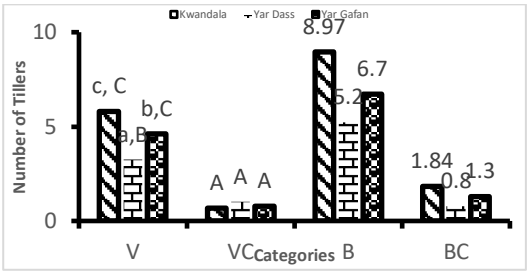

Figure 3: Effect of flood stress on the number of leaves in three rice genotypes

Values are presented in mean ± standard deviation of three independent determinations. Values in the same group followed by different lowercase letters are significantly different (P<0.05). Values in the different categories followed by different uppercase letters are significantly different. VC: Vegetative control, V: Vegetative, B: Booting, BC: Booting control.

Biochemical Test

α-Tocopherol: The α-tocopherol content of three rice genotypes was analyzed as shown in Table 1. The α tocopherol content ranges between 0.093 and 1.07 (mg/dL) at the vegetative stage. The result showed that Kwandala had the lowest level of α-tocopherol content (0.093 mg/dL), followed by Yar-gafan (0.65 mg/dL), and finally, Yar-dass with the highest level of α-tocopherol (1.07 mg/dL). At the booting stage, α Tocopherol content ranges between 4.87 and 10.74 (mg/100g). The result showed that Yar-gafan had the lowest level of α-tocopherol content (4.87 mg/100g), followed by Yar-dass (8.61 mg/100g), and Kwandala with the highest level of α-tocopherol content (10.75 mg/100g). There was a statistically significant difference (P <0.05) between the genotypes tested.

Table 1: α-Tocopherol content of three rice genotypes

GENOTYPE	VEGETATIVE (mg/dL)	BOOTING (mg/100g)
KWANDALA	0.093±0.014^{a}	10.749±2.424^{c,*}
YAR-DASS	1.071± 0.1252^{c}	8.613±2.697^{b,*}
YAR-GAFAN	0.652± 0.171^{b}	4.886±1.069^{a,*}

Values are presented as mean ± standard deviation of three independent determinations.

Values in the same column followed by different lowercase letters are significantly different (P<0.05). Values in the same row followed by * indicate a significant difference (P<0.05).

Ascorbate: The ascorbate content of three rice genotypes was analyzed as shown in Table 2. The ascorbate content ranges between 3.66 and 7.65 mg/dL at the vegetative stage. The result showed that Kwandala had the lowest level of ascorbate content (3.66 mg/dL), followed by Yar-dass (5.32 mg/dL), and finally, Yar-gafan with the highest level of ascorbate (7.65 mg/dL). At the booting stage, ascorbate content ranges from 4.12 to 11.12 mg/100g. The result showed that Yar-dass had the lowest level of ascorbate content (4.12 mg/100g), followed by Kwandala (5.90 mg/100g), and Yar-gafan with the highest level of ascorbate content (11.12 mg/100g). There was a statistically significant difference (P <0.05) between all the genotypes tested.

Table 2: Ascorbate content of three rice genotypes

GENOTYPE	VEGETATIVE (mg/dL)	BOOTING (mg/100g)
KWANDALA	3.656±1.654^{a}	5.901±1.305^{a,*}
YAR-DASS	5.318±2.556^{a}	4.106 ± 1.678^{a}
YAR-GAFAN	7.649±2.156^{c}	11.117±3.498^{b,*}

Results are mean ± standard deviation of three independent determinations. Values in the same column followed by different lowercase letters are significantly different (P<0.05). Values in the same row followed by (*) indicate a significant difference (P<0.05).

Glutathione: The glutathione content of three rice genotypes was analyzed as shown in Table 3. The glutathione content ranges between 7.53 and 10.313 mg/dL at the vegetative stage. The result showed that Yar-dass had the lowest level of glutathione content (7.530 mg/dL), followed by Yar-gafan (7.592 mg/dL), and finally, Kwandala with the highest level of glutathione (10.313 mg/dL). At the booting stage, glutathione content ranges between 41.77 and 42.350 (mg/100g). The result showed that Yar-dass had the lowest level of glutathione content (41.770 mg/100g), followed by Yar-gafan (41.933 mg/dL), and Kwandala with the highest level of glutathione content (42.350 mg/100g). There was a statistically significant difference between all the genotypes tested

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Table 3: Glutathione content of three rice genotypes

GENOTYPES	VEGETATIVE (mg/dL)	BOOTING (mg/100g)
K	10.313±2.79^{b}	42.350±4.419 *
D	7.530±1.491^{a}	41.770±3.795*
G	7.952±1.634^{a}	41.933±8.951 *

Values are mean ± standard deviation of three independent determinations. Values in the same column followed by different lowercase letters are significantly different (P<0.05). Values in the same row followed by (*) indicate significant differences (P<0.05) between the groups.

MDA: The lipid peroxidation of three rice genotypes was analyzed as shown in Table 4. The MDA content ranges between 2.2±0.7 to 3.6±0.62 (μmolg^{-1}) for treatment at the vegetative stage, Kwandala having the lowest content (2.2±0.7), followed by Yar-dass (2.7±0.5) and Yar-gafan with the highest (3.6±0.62), while the control group at the vegetative stage, the MDA content ranges between 0.81±0.19 and 0.95±0.33 (μmolg^{-1}), Kwandala having the highest content (0.95±0.33), followed by Yar-gafan (0.90±0.20) and Yar-dass with the lowest content (0.81±0.19). However, The MDA content ranges between 3.96±1.34 and 5.05±0.30 (μmolg^{-1}) for treatment at booting stage, Kwandala having the lowest content

(1.32±0.49), followed by Yar-dass (3.96±1.34) and Yar-gafan with the highest (5.05±0.30) while the control group at booting stage, the MDA content ranges between 0.21±0.033 and 0.90±0.03 (µmolg^{-1}), Kwandala having the lowest content (0.21±0.33), followed by Yar-dass (0.70±0.20).Yar-gafan with the highest content (0.90±0.03).

Table 4: Malondialdehyde content of three rice genotypes

GENOTYPES	V (µmolg^{-1})	Vc (µmolg^{-1})	B (mg/100 g)	Bc
K	2.2±0.70^{a,*}	0.95±0.33	1.32±0.49^{b,*}	0.21±0.03^{a}
D	2.7±0.50^{a,*}	0.81±0.19	5.05±0.30^{b,*}	0.70±0.20^{b}
G	3.6±0.62^{b,*}	0.90±0.20	3.96±1.34^{b,*}	0.90±0.03^{b}

Results are presented in mean ± standard deviation of three independent determinations. Values in the same column followed by different lowercase letters are significantly different (P<0.05). Values in the same row followed by (*) indicate significant differences (P<0.05) between the groups. VC: Vegetative control, V: Vegetative, B: Booting, BC: Booting control.

PCR Products

Three rice genotypes were used: Kwandala, Yar Gafa and Yar-dass. Seeds of all the genotypes amplified with the SK1 and SK2 SNP markers. A single rice genotype gave a PCR product of ~949 bp (plate 1) for SK1 (Kwandala) and two rice genotypes gave a PCR product of ~184 bp for the SK2 (Yar Gaffan and Kwandala) gene, respectively.

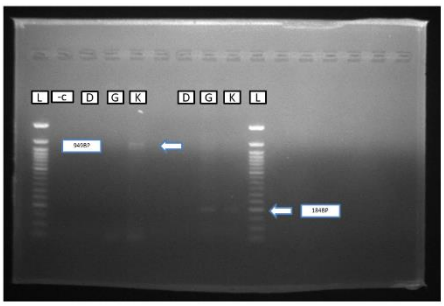

Plate 1: Electropherogram amplicon of SK1 and SK2 SNP markers on three rice genotypes (Kwandala, Yar-gafan, and Yar-dass).
First and last lane are the DNA ladder, one genotype showed 949 bp band size for SK1, two genotypes showed 184bp band size for SK2. L=50 bp ladder [New England Bio Lab (50-1,350 bp)]. L stands for ladder.

Phylogenetic Analysis

The SK1 sequence of Kwandala was clustered into two main groups. The first group comprised a single accession (LC201746.1) in Figure 4. The second group comprised four clusters; a divergence is relatively seen among the clusters, Kwandala shares the same cluster with the accession AB510480.1, showing no divergence. In Figure 5, sequences from the two rice genotypes (Yar-gafan and Kwandala) share the same cluster, highlighting no divergence, while other accessions (AB510481.1, AB510479.1, and AB510483.1) are distinctly apart, exhibiting a moderate level of divergence.

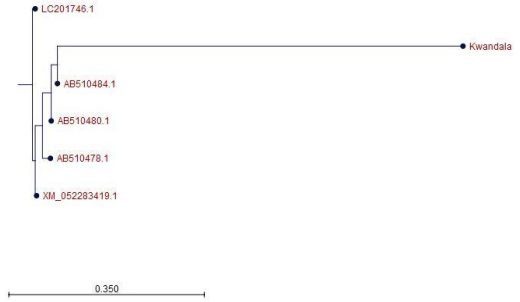

Figure 4: Maximum likelihood tree of SK1 sequence of Kwandala with other varieties in the public database after blast search with over 60% bootstrap, accessions having closer distances are genetically similar.

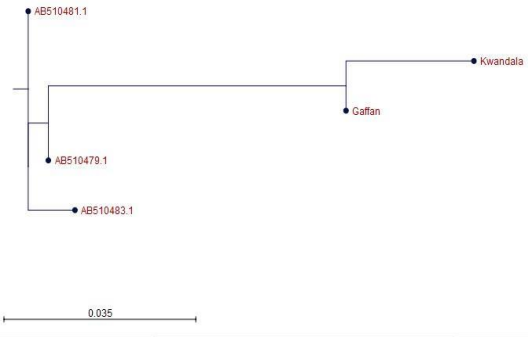

Figure 5: Maximum likelihood tree of SK2 sequence of Yar-gafan and Kwandala with other varieties in the public database after blast search with over 60% bootstrap, accessions having closer distances are genetically similar.

DISCUSSION

In the present study, rice development was greatly affected by stress imposed at the vegetative stage rather than the reproductive stage. Survival of rice seedlings after complete submergence in water decreased with increased duration of submergence, while their survival increased with light intensity and plant carbohydrate, as established by Palada and Vergara (1972).

Tolerance of rice to flood stress via snorkeling strategy at the vegetative stage is significantly lower across the genotypes compared to the reproductive stage. This might be attributed to the quiescence strategy that plays a key role in attaining submergence tolerance at the vegetative stage and not the snorkeling as established by Yamauchi *et al.* (1993). Among the three rice genotypes, Kwandala emerged with the highest score with regard to plant height and has both SK1 and SK2 genes after DNA extraction and PCR test.

Plant height during stress studies in vegetative and reproductive phases revealed the genotypes that employ the snorkeling strategy as a coping mechanism. As such, the growth of plant height under submergence can be an indicator of a flood-stress tolerant trait. The observed differences in plant height between the three rice genotypes are likely linked to the development of critical anatomical traits for adaptation to anaerobic conditions, such as aerenchyma formation, this was reported by Phule *et al*. (2019), while the presence of the SNP markers (SK1 and SK2) in rice genotypes might be responsible for shoot elongation (Hattori *et al*., 2009).

The number of leaves was the same with the number of tillers in both vegetative and booting stages, this is due to the method of planting employed during the research, as reported by Jagtab *et al*. (2018) that crop establishment method and fertilizer sources are key factors that cause variations in plant height, number of leaves, leaf plant area and number of tillers, no fertilizer was applied during the study and no transplanting took place. Plant cells can neutralize the ROS with efficient oxygen-scavenging machinery consisting of several antioxidants, both non-enzymatic and enzymatic (Gill and Tuteja, 2010). In the present study, tolerant cultivars have a greater quantity of ascorbate, glutathione, and tocopherol during submergence, as they play an important role in limiting the extent of damage caused by ROS. All three rice genotypes showed a considerable amount of non-enzymatic antioxidants, which is considered a resistant trait as hinted by Saikia *et al*. (2021).

MDA is one of the products of plant lipid peroxidation, resulting from oxidative stress-induced damage to the membrane (Guo *et al*., 2007). As established by Damanik *et al*. (2012), a higher content of MDA signifies a receptive trait, while a lower content indicates a resistant trait; hence, the variety Yar-gafan is a receptive trait, while Kwandala is a resistant trait. In the present study, the MDA content was found to be significantly higher in genotypes with low snorkeling activity; this is due to higher damage caused by ROS. The MDA content is found to be higher in all the cultivars during submergence compared to the control group across the three genotypes, as an indication that the control group is not undergoing the flood stress. A similar result was reported by Panda *et al*. (2013).

Plants have developed various organs, forms of stress tolerance, and environmental adaptability by increasing their genome sizes during evolution. The Increase in functional diversity is a result of whole genome duplication or segmental, tandem, and transposon-mediated gene duplications (Cannon et al., 2004). The phylogenetic tree highlighted the divergence and variance of the selected genotypes and other rice genotypes regarding the snorkel gene (SK1 and SK2).

CONCLUSION

In the present study, flood stress had a significant impact on rice at the vegetative and booting stages of growth. The morphology was also affected by flood depth, and the duration of the flood affects the process of de-submergence. The oxidant and antioxidant content of rice in treatment and control explained the stress level of rice varieties under treatment. Rice varieties with the SNP markers deployed the snorkeling strategy as a coping mechanism. Kwandala emerged as the best variety amongst the three local rice genotypes to tolerate flood stress. Flooding affected the growth of the three local rice genotypes across their developmental stages.

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