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Response of *Oryza glaberrima Steudel* to Salt Stress at Seedling Stage

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Salt stress is one of the major environmental challenges that limit rice production globally. As climate change exacerbates this issue, the development of salt-tolerant rice varieties has become crucial for sustaining food security. This study evaluated the salt tolerance of Oryza glaberrima genotypes and explored the genetic associations of salt tolerance using tagged SSR markers. The research was conducted under hydroponic conditions at the Department of Botany, Ahmadu Bello University, Zaria, using standard methods. Forty-three Oryza glaberrima genotypes were studied. The genotypes were sown in styrofoam floats suspended in 15-liter, four-cornered, dark-coloured plastic bowls. After seven days, 1 g of FeSO4 and 10 g of Peter's 20-20-20 water-soluble fertilizer were added to the nutrient solution. Subsequently, NaCl, FeSO4 and fertilizer were added to simulate saline conditions. Plant biometrics were evaluated, and DNA was extracted from young leaf samples. PCR amplification with seven SSR primers associated with salt tolerance revealed significant genetic variation among the genotypes. The response to salt stress was genotype-dependent, with approximately 14% of the genotypes showing tolerance or moderate tolerance, while 86% were highly susceptible to salt stress. The susceptible genotypes exhibited severe visual symptoms of salt injury. Two distinct genetic clusters were observed based on the SSR markers, with the majority of the susceptible genotypes (97.3%) clustering in one group, except for TOG14101, which clustered separately. The study confirmed that SSR markers are effective tools for distinguishing between salt-tolerant and susceptible Oryza glaberrima genotypes, providing valuable insights for breeding programmes.

Keywords: *Oryza glaberrima*, salt tolerance, salinity, seedling stage.

1. Introduction

A saline soil is characterized by a high concentration of soluble salts that can negatively impact plant growth. The salt concentration in soil is measured through its electrical conductivity, and high soil salinity can limit rice production (Mohammed-Nejad et al., 2008; Aliyu, 2016). Salinity affects rice growth at all stages, from germination (Aliyu, 2016; Aliyu and Sakariyahu, 2016) to maturation (Maas and Hoffman, 1977; Aliyu *et al*., 2016). The seedling stage is particularly sensitive and crucial, as it significantly influences crop establishment (Singh et al., 2008; Aliyu and Sakariyahu, 2016). A comparison of salt tolerance during germination and emergence with that during succeeding growth stages showed that while rice was very tolerant of salinity during the germination process, it was very sensitive during the first to second leaf stages (Aliyu, 2016). Salinity reduces the growth of plant through osmotic effects, reduces the ability of plants to take up water and this causes a reduction in growth. There may be salt specific effects. If excessive amount of salt enters the plant, the concentration of salt will eventually rise to a toxic level in older transpiring leaves causing premature senescence and reduces the photosynthetic leaf area of a plant to a level that cannot sustain growth (Munns, 2002). Gregorio *et al*. (1997) emphasized that salinity symptoms were prominent on the first and second leaves and were visualized by leaf rolling, formation of new leaf, brownish and whitish leaf tip, drying of leaves and also reduction in root growth, stunted growth and stem thickness leading to complete cessation of growth and dying of seedlings. Extremely high salt stress conditions cause severe damage to plants, while moderate to low salt stress affects the plant growth rate along with most of the growth and yield parameters (Aliyu *et al*., 2016). Accurate phenotypic identification of tolerant lines is difficult because of the extensive effect of the environment on salt tolerance (Singh *et al*., 2008). This has necessitated the use of hydroponic culture solutions for salinity tolerance screening for crops such as rice (Gregorio et al., 1997).

Oryza glaberrima, a species of rice is endemic to Africa (Linares, 2002). It is indigenous to Nigeria and has been cultivated for the past 3,500 years (Hardcastle, 1959). The earliest cultivation of improved rice varieties (*O. sativa* L.) started in about 1890 with the introduction of upland varieties to the high forest zone in Western Nigeria (Atanda *et al*., 1978). Consequently, by 1960 *O. sativa* had taken over from *O. glaberrima*, which is now limited to some deep-flooded plains of the Sokoto-Rima River basin and other isolated pockets of deep swamps all over the country (Imolehin, 1991). Although both species exhibit significant polymorphism in grain and other structures (Muhammad *et al*., 2024), farmers in these regions prefer *Oryza glaberrima* due to its tolerance to various biotic and abiotic stresses. (Aliyu, *et al*., 2016; Azeez, *et al*., 2016; Prodjinoto, *et al*., 2018; Indabo, *et al*., 2020; Muhammed, *et al*., 2022). Thus, the need for its preservation through cultivation alongside the Asian species as soils in arid/semi-arid regions show some degree of salinity.

The increasing salinization of soils due to both natural processes and human activities poses a significant threat to global rice production. While much research has been focused on the salinity tolerance of *Oryza sativa* (Alam *et al*., 2004; Aliyu, 2016), less attention has been given to *Oryza glaberrima*, a species known for its potential resilience in saline conditions. This study aimed to screen *Oryza glaberrima* genotypes for their response to salt stress at the seedling stage, a critical phase for rice establishment. By identifying salt-tolerant genotypes, this research provides valuable data that can guide the development of more robust varieties of rice suitable for cultivation in saline-prone areas. The findings from this study are particularly important for enhancing food security in regions where salinity is a major constraint to agricultural productivity. Furthermore, this work contributes to the growing body of knowledge on the genetic potential of *Oryza glaberrima* in adapting to harsh environmental conditions, which could play a pivotal role in the face of global climate change.

2. Methodology

The study was conducted at the glass house of the Department of Biological Sciences, Ahmadu Bello University, Zaria. Forty-three genotypes used for the study were obtained from AfricaRice, Ibadan Station, Nigeria.

Rice seeds were directly sown at a rate of two per hole in the Styrofoam float suspended on a 15litre four-corner dark coloured plastic bowl. After germination, the seedlings were thinned to one plant per hole. Seven days after seeding emergence, one gram of FeSO4 and 10g Peter's 20-20-20 water soluble fertilizer were added to the distilled water in the plastic tank. The nutrient bowl (15- liter capacity) were filled with the hydroponic solution high enough to touch the rubber net bottom of the Styrofoam. Eight days after seeding, 50g of NaCl was used for salinization (equivalent to 12 dS/m). The pH was maintained at 5.0 ± 0.1 using 1N HCl and 1N NaOH by utilizing sensitive paper strips. Due to evaporation and transpiration, the loss of solution volume in the bowls was made up with water daily. One gram of $FeSO_4$ was added weekly and 10g of Peter's water-soluble fertilizer fortnightly to maintain the nutrient concentration in solution.

The study was conducted in a completely randomized design with three replicates and control checks (1R29 susceptible and Pokkali tolerant). Salinized and non-salinized hydroponics were maintained for 30 days. Salinity symptoms were scored using the standard evaluation system (IRRI, 1997).

Seedling height (cm) and leaf blade colour were taken twenty-eight days after salinization. Root length (RL) (cm) was measured and recorded. Root dry weight (RDW) (mg) and shoot dry weight (SDW) (mg) and Leaf number (LN) were taken.

Quantitative data were analyzed using Analysis of variance (ANOVA) and mean separation using Duncan Multiple Range Test (DMRT). This was performed using the GLM procedure of Statistical Analysis System (SAS Institute Incorporation, 1999).

2.1 DNA extraction and SSR markers

Genomic DNA was extracted from young leaf tissues following the method described by Aliyu et al (2016), using 5 mg of leaf materials from at least 3 seedlings. Quantified DNA from each genotype was subjected to PCR amplification with 7 SSR primers (RM17, RM240, RM286, RM493, RM495, RM10711 and RM10793) tagged to salinity tolerance (Aliyu et al., 2013; Aliyu and Adamu, 2023). For each marker, allelic bands were scored on a 1 (present) and 0 (absent) binary codes. Only prominent and unambiguous bands were scored for data reliability. Genetic similarities were evaluated using the single data analysis for neighbor joining analysis of the DARwin 5 version 5.0.157 software.

3. Results and Discussion

Phenotypic biometrics of forty-three (43) *Oryza glaberrima* genotypes screened for salt tolerance at EC 12 dS/m with the tolerant- (Pokkali) and susceptible (IR29) checks as well as the response of *Oryza glaberrima* to salt injury at seedling stage based on their phenotype and standard evaluation score (IRRI, 1997) are presented in Table 1.

Table 1. Phenotypic biometrics of forty-three (43) O*ryza glaberrima* genotypes screened for salt tolerance at ec 12 ds/m with the tolerant- (pokkali) and susceptible (ir29) checks column values followed by the same letters are not significantly different using duncan's multiple range test.

S/N	GENOTYPE	SH	G. RATE (%)	TN	RL	RDW	SES SCORE
1	IR29	13.73cj	42.6hi	3.00a	7.33bf	0.43fj	9d
2	POKKALI	28.87a	74.5fg	3.00a	8.51af	1.93a	1a
3	TOG12405	22.6ac	131.3cd	2.67ab	11.93ae	1.09bi	5c
4	TOG12303	20.73ad	92.5e	3.00a	12.5ae	0.77bj	9d
5	TOG12373	19.40bf	85.3ef	3.00a	11.15af	1.33bc	3b
6	TOG12407	21.65ad	98.6de	3.00a	13.7ab	1.23bd	5c
7	TOG12444	17.73bg	92.1e	2.67ab	11.00af	0.60dj	9d
8	TOG14101	6.97ik	15.6j	2.00bc	8.93af	0.57dj	9d
9	TOG5282	19.87be	82.3ef	3.00a	13.9ab	1.13af	9d
10	TOG5284	13.80cj	114.0d	3.00a	12.65ad	0.63cj	9d
11	TOG5313	19.77be	70.9g	3.00a	12.5ae	0.53dj	9d
12	TOG5314	10.47gk	77.5f	2.67ab	9.07af	0.30fj	9d
13	TOG5321	9.95gk	134.1cd	3.00a	7.45bf	0.40ej	9d
14	TOG5406	14.60bj	88.4ef	3.00a	12.15ae	0.50dj	9d
15	TOG5418	4.4k	44.3h	1.50c	8.50af	0.17hj	9d
16	TOG5456	6.7ik	370.0a	1.50c	4.8f	.13ij	9d
17	TOG5331	12.65ek	87.ef	3.00a	13.1ac	1.2ac	5c
18	TOG5457	20.22bd	347.6b	2.80ab	12.12ae	0.77bj	9d
19	TOG5479	19.73be	132.1cd	2.67ab	11.63af	0.63cj	9d
20	TOG5508	8.2hk	37.4i	2.00bc	4.85f	0.13ij	9d
21	TOG6165	13.45dj	138.1cd	2.00bc	10.15af	0.17hj	9d
22	TOG6193	6.2jk	69.9fg	2.00bc	6.5fce	0.23fj	9d
23	TOG 6600	10.55gk	75.8f	2.50ab	9.40af	0.07j	9d
24	TOG6762	16.30dh	132.9cd	2.67ab	14.67a	1.5ac	9d
25	TOG6785	6.4jk	66.2f	2.00bc	5.75ef	0.07j	9d
26	TOG7134	17.40bg	118.9d	2.67ab	10.40af	0.80bj	9d
27	TOG7191	17.10bg	121.2cd	3.00a	13.13ac	1.37ad	9d
28	TOG7390	11.10ek	68.2fg	2.33ac	6.7fce	0.23fj	9d
29	TOG7401	14.30cj	56.0g	3.00a	12.73ad	0.67cj	9d
30	TOG7426	19.83be	73.2fg	2.33ac	6.33fe	0.43ej	9d
31	TOG7427	17.20bg	164.6c	2.33ac	12.63ad	0.87bj	9d
32	TOG7428	21.3ad	95.9cd	3.00a	10.07af	1.07bh	5c
33	TOG7742	23.47ab	83.8ef	3.00a	12.93ad	1.57ab	9d
34	TOG7943	15.27bi	71.6fg	2.67ab	10.33af	0.73bj	9d

35	TOG7992	20.2bd	99.4de	3.00a	15.07a	1.13af	9d
36	TOG8006	15.95bh	109.9de	2.5ab	8.50af	0.40ej	9d
37	TOG8008	21ad	102.0de	3.00a	13.9ab	0.60gj	9d
38	TOG8044	18.75bg	92.3e	3.00a	7.7fe	0.07j	9d
39	TOG9047	17.87bg	105.4de	2.67ab	8.77af	0.80bj	3b
40	TOG9276	6.65ik	137.5cd	1.50c	5.65ef	0.17hj	9d
41	TOG9278	14.67bj	49.2h	3.00a	13.83ab	1.27ae	9d
42	TOG9281	23.37ab	82.2ef	3.00a	14.93a	1.33ad	9d
43	TOG9395	17.33bg	67.1fg	2.67ab	9.80af	1.10ag	9d
44	TOG9396	10.80gj	66.2f	3.00a	7.5fbe	0.23fj	9d
45	TOG9524	18.57bg	70.8fg	3.00a	11.50af	1.03bi	9d
	C.V	16.21	12.22	14.94	19.5	18.83	
	R^2	0.76	0.77	0.66	0.78	0.74	
	STDEV	6.44	3.63	0.53	3.81	0.64	
	MIN	1	17.6	1	1.03	0.07	
	MAX	31.6	570	3	20.6	1.93	
	P VALUE	<.01	<.01	<.05	<.01	<.01	

Key: C.V- Coefficient of Variance, SH- Seedling Height, G. RATE- Growth Rate, TN- Tiller number, RL- Root Length, RDW- Root Dry Weight, SES- Standard Evaluation system

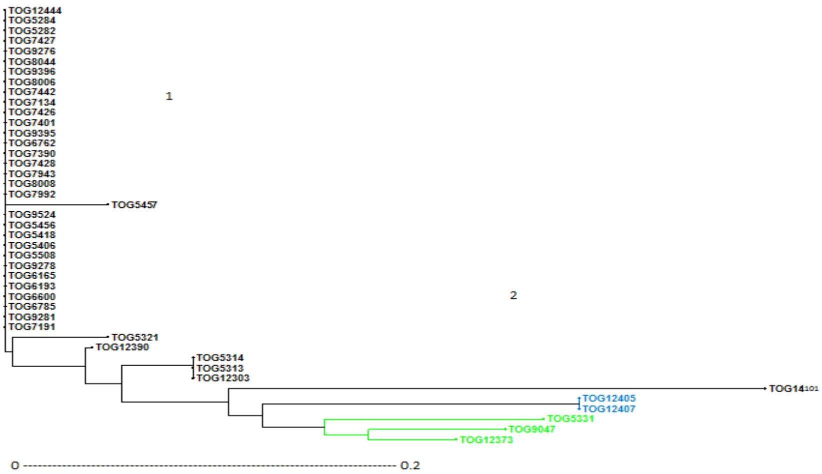

Figure 1. Dendrogram of 43 *Oryza glaberrima* genotypes (green colour – tolerant, blue colour- moderately tolerant) based on 7 salts tolerant tagged Polymorphic SSR Markers Derived from UPGMA Cluster Analysis using DARwin5 software (version 5.0.157).

The genotypes showed varied visual symptoms of salt injury in salinized conditions. A ratio of approximately 1:6 tolerant and moderately tolerant genotypes (14%) to highly susceptible genotypes (86%) at an electrical conductivity (EC) of 12dsm-1 was observed. Six moderately tolerant (TOG5331, TOG7428, TOG12405 and TOG12407) and tolerant (TOG9047 and TOG12373) genotypes were obtained. These tolerant genotypes (TOG9047 and TOG12373) were comparable to the highly tolerant genotype (Pokkali). Nine genotypes TOG5314, TOG5321, TOG5508, TOG6600, TOG6785, TOG7390, TOG7428, TOG8044 and TOG9396 were more susceptible than IR29. Some degree of cultivar tolerance to salinity and other abiotic stress is available in rice germplasm and have been reported in several research (De Datla et al., 1993, Gregorio, 1997, Aliyu and Sakariyahu, 2016; Muhammed, et al., 2022). Akbar et al. (1987) and Aliyu et al. (2016) further reported that African O. glaberrima screened at 2-3 leaf stage at EC 12 dsm-1 for 14 days were highly susceptible at seedling stage with a survival rate of 0-16% while the tolerant control nona Bokra showed complete survival.

The interactions between salinity and genotype were also significant (P<0.01) for plant growth parameters. Variations in seedling heights were observed for all genotypes. The seedling heights were shorter in salinized conditions compared to plants grown in non-salinized conditions. Seedling heights of susceptible genotypes also showed higher percentage reductions than those of tolerant genotypes. Majority of the moderately tolerant to tolerant genotypes showed a decline in growth rate (computed with reference to non-saline treatment) ranging from 4.1% to 25.4%. Decline in growth rate for susceptible genotypes ranged from 15.65 to 570%. Tillering ability was not significantly affected by salinity stress. Most genotypes showed no significant difference in tillering ability under salinized conditions. Root length was significantly ($p<0.05$) affected by salinity. Salinity did not affect the root architecture of some genotypes. In others, varied root length retardation of 8.51cm in Pokkali, 8.77 cm in TOG9047 to 13.7cm in TOG12407 amongst tolerant genotype were observed. General reductions in the root dry weight across all genotypes were obtained.

Salt tolerant genotypes had reduced plant growth rate which implied a reduction in energy utilization thus conserving energy by maintaining low growth rate and consequently lower biomass production. Susceptible genotypes were characterized by their inability to absorb salt in water probably as a result of the toxic ions sneaking into the plant along with the water stream which moves to the vascular system of the root by the symplastic and apoplastic pathways (Garciadebleas et al., 2003. Tolerant genotypes showed lower reductions in characterized traits than in susceptible ones. Seedling height was shorter in susceptible than tolerant genotypes indicating that salinity stress affect the seedling height of the genotypes by interfering with growth mechanisms thereby affecting the photosynthesizing abilities of these genotypes. On the other hand, the tiller numbers produced by some tolerant and susceptible genotypes were higher in salinized treatments. Increased tillering ability in salinized conditions in some genotypes might be due to the genotypes tillering ability and vigour. Alternatively, it might have been due to unclear morphological determinants which might have triggered some mechanisms that triggered the genotypes to respond more vigorously in the bid to escape long exposure to the stress factor. Increased tillers have also been reported by Afza et al. (2009) in their work on double haploid and induced mutation in breeding salt tolerance in rice and wheat. Reduction in tillering ability of susceptible genotypes was also observed. This result was in accordance with the report of Farah and Anter (1978); Aliyu (2016) observed that salinity decreased tillering in sensitive rice than in tolerant genotypes. The reductions in the growth parameters observed in the genotypes are in line with report from previous investigations (Aliyu et al., 2016) At salinity levels as low as 1.9 dS/m, seedling growth is significantly reduced. Munns (2002) reported that many rice cultivars suffer a 50% reduction in growth at 7.5 dS/m.

A significant positive association ($r=0.61$) was observed between root length and root dry weight. In susceptible genotypes root dry weight obtained was as low as 0.07 g and as high as 1.57 g. Root dry weight (1.93 g) was highest in Pokkali. TOG9047 and TOG12373 had root dry weight of 0.80 g and 1.33 g respectively. It had been reported that salinity caused some morphological changes like reduction of shoot (Mishra et al., 1995), root length (Evers et al., 1997; Aliyu et al., 2016) and restriction of rooting (Lopez and Satti. 1996). Munns et al. (1995) also reported that, salinity might directly or indirectly inhibit cell division and enlargement in plant's growth phases. Alam et al. (2004) attributed the possible reasons for decrease in the root growth in salinized plants as reduction of photosynthesis, which in turn limits the supply of carbohydrates needed for growth and reduction of turgor in expanding tissues resulting from lowered water potential in root growth medium. Plasticity due to salinity stress in susceptible genotypes was observed with some growth parameters (increased root length, root dry weight, and tillering ability). This could be attributed to the initial vigour of these genotypes in the bid to escape unfavourable condition as they compete for nutrients.

Two cluster groups (Figure 1) were obtained on the dendrogram constructed with seven SSR markers based on neighbour joining analysis to characterize tolerant and susceptible genotypes. Most of the susceptible genotypes (97.3%) clustered together in Cluster group 1 except for TOG14101 which out clustered in group 2. The moderately tolerant to tolerant genotypes (83.3%) clustered in group 2 except for TOG7428 that clustered in group 1. Genetic diversity and the relationship between and among species are of great importance for rice breeding. Genotypes derived from genetically similar types clustered together as species exhibited a spatial structure of genetic variation.

This was supported by works of Ge et al. (1999), Joshi et al. (1999) and Ren et al. (2003). The different level of diversity may be attributed to the rate of mutation, migration, dispersal mechanisms, biotic and abiotic selection intensities which are determined by location, climate and soil as also reported by Kark et al. (1999). Tagged molecular markers to abiotic stress could be utilized in the profiling of salt tolerant lines within and between species (Aliyu et al., 2013; Aliyu and Adamu, 2023). The ability of the tolerant genotypes to cluster together was as a result of selection pressure by the tagged markers.

4. Conclusion

Conclusively, 6.98% of *Oryza glaberrima* genotypes showed tolerance to salinity. TOG5331, TOG9047 and TOG12373 are considered potential salt tolerant *Oryza glaberrima* genotypes. Tagged SSR markers were 83.3% efficient in identifying salt tolerant *Oryza glaberrima* genotypes.

Authors' Contributions

ARE and AJO conceptualized and designed the study, performed the statistical analysis and wrote the first draft of the manuscript. HUM, SKS, SSI, AA, AOA improved the manuscript, AAK proof read and effected corrections to the manuscript. All authors approved the final manuscript."

Conflict of Interest

The authors declare no conflict of interest.

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