Rice Research: Open Access
ISSN: 2375-4338

Mayaki, et al., J Rice Res 2025, 13:1

Research Article Open Access

Assessment of Some Rice (*Oryza sativa* L.) Genotypes for Salinity Stress Tolerance Using Morpho-Physiological and Molecular Analysis

Bashir Mohammed Mayaki[1*], Lawan Abdu Sani[1], Usman Abubakar[1], Jibril Sani Mohammed[2], Nuradeen Muhammed[1] and Rukayya Kabir Mato[1]

[1]*Department of Plant Biology, Bayero University, Kano, Nigeria*

[2]*Department of Agricultural Biotechnology, Biological Institute, National Research Tomsk State University, Tomsk, Russia; Department of Plant Biology, Faculty of Life Science, Federal University, Dutse, Nigeria*

Abstract

The rice plant is salt sensitive and its productivity is severely affected by the accumulation of soluble salts in soils. Salinity is an ever increasing problem that reduces rice yield in rice producing areas. The arid and semi-arid zone of Nigeria where irrigation practices is widely adapted faces the most serious ecological and environmental problems ensuing from high saline soil conditions. Identification of salt tolerant rice genotype is one of the solutions to the problem of salinity. A study was conducted under a controlled environment in the department of plant biology and centre for dry land agriculture of Bayero University Kano, to evaluate the responses of six rice genotypes at four levels of NaCl concentrations (0 dS/m, 4 dS/m, 8 dS/m and 10 dS/m). Data were collected on plant height, leaf chlorophyll content, shoot fresh and dry weight, root fresh and dry weight, tiller per plant, panicle length, panicle number grain yield per pot and 1000 grain weight and were subjected to analysis of variance for mean comparison. The result of analysis of variance revealed that the parameters measured were negatively affected for all varieties at different concentrations except at (0 dS/m) which is the control. The most effect was observed particularly in the range of 8 to 10 dS/m. FARO 44 and FARO 67 outperformed other varieties with respect to the data collected as they appeared to be moderately tolerant to the induced stress. This could be attributed to their inherent genetic characteristics. Four candidate genes (*OsMYB6*, *OsGAMYB*, *OsHKT1;4*, and *OsSUT1*) reported to contribute to salinity tolerance in rice were selected and identified in some of the studied rice varieties. The presence of all four genes were detected in *FARO 44* and *FARO 67*, these genes are believed to have contributed to their outstanding performance under the salinity stress thereby contributing to their tolerance potential, although the genes were also present in some of the susceptible varieties, their expression might have been suppressed hence their poor performance under the salt stress.

Keywords: Rice; Salinity; Electrical conductivity; Candidate genes; Sodium chloride

Introduction

Abiotic stresses such as salinity, drought and extreme temperatures have a crucial impact on agricultural productivity and yields [1]. Saline soil condition is a common abiotic stress that reduces productivity in many rice producing areas. The production of rice being a salt sensitive crop is considerably affected by salinity, which has been recognized as the second most widespread soil problem in rice growing countries, after drought. Rice growing countries, both in the tropics and the temperate regions, are facing high soil salinity as a major problem which is more severe in the arid, semiarid, and coastal rice producing areas of the tropics [2].

The extent of sensitivity to salt stress in rice plant varies upon the plant growth stage with most cultivars recording more damage at germination and early seedling stage, indicating that salinity has negative effect on the plant early growth stages [3]. It also affects the, plant growth, development, root index, root length shoot length and ultimately productivity. Salt affected soils have much accumulation of complex combination of soluble salts. However, NaCl is considered the main cause of soil salinization, because of its abundance in many affected soils and its high solubility.

The effect of high salinity on plant can be detected at the whole plant level in terms of decline in productivity and ultimately plant death. According to a global projection salt affected soils are reported to be rising particularly in irrigated areas from 20% (45 million hectares) to 33% (74.25 million hectares) in the last decades [4].

The increase recorded in the number of irrigated areas suggest that at a global scale, an area of about 2000 ha of irrigated cropland is affected by varying levels of salinity daily [5]. Jamil et al., reported that it has been estimated that more than 50% of the arable land would be salinized by the year 2050. The main sources of salts include rainfall, mineral weathering, irrigation and various saline water bodies, underground water which redistributes accumulated salts during evaporation, chemical fertilizers applications, and man activities [6].

Na^+ and Cl contained in some irrigation waters have detrimental effects on the physical properties of soils due to its connection with the accumulation of sodium ion on the soil exchange complex. This ultimately disrupts the stability of the soil aggregates resulting into the dispersion of soil particles, clogging of soil pores and the destruction of crops. Tully's study as cited in expressed that soluble salts are drawn to the soil surface by evapo-transpiration from underground

***Corresponding author:** Bashir Mohammed Mayaki, Department of Plant Biology, Bayero University, Kano, Nigeria; E-mail: mayakibashir@gmail.com

Received: 30-September-2023, Manuscript No. RROA-23-115430; **Editor assigned:** 02-October-2023, PreQC No. RROA-23-115430 (PQ); **Reviewed:** 16-October-2023, QC No. RROA-23-115430; **Revised:** 22-January-2025, Manuscript No. RROA-23-115430 (R); **Published:** 29-January-2025, DOI: 10.4172/2375-4338.1000449

Citation: Mayaki BM, Sani LA, Abubakar U, Mohammed JS, Mohammed N, et al. (2025) Assessment of Some Rice (*Oryza sativa* L.) Genotypes for Salinity Stress Tolerance Using Morpho-Physiological and Molecular Analysis. Environ Pollut Climate Change 13: 449.

water forming white crusts clearly indicating the presence of salinity. Farming in such an area is severely impacted by this process.

In Nigeria, rice is much consumed in almost all homes, the share of rice in cereals consumed increased from 15% in the 1970's to 26% in the early 1990's [7]. Despite the increase in the total rice production, the rising population combined with climatic changes poses a great threat to its production. The prevalent types of rice production systems in Nigeria include rainfed upland, rainfed lowland and irrigated lowland with each type having specific characteristics and production potential depending on varying soil and climatic conditions. Globally, it is estimated that 19.5% of irrigated land (230 million ha) and almost 2.1% of dry land agriculture (45 million ha) is affected by salt [9]. In the arid and semi-arid zone of Nigeria, salt salinity is a serious problem causing considerable loss in the growth and productivity of rice plant. The effect of salinity is more in rice plant during the early growth stage and reproduction stage. Therefore, the need to evaluate seedling based and yield characters to identify and screen the best varieties that are tolerant to salinity conditions. Identification of rice strains that tolerate and thrive in high salt concentrations will allow inhabitants in these high saline areas to also thrive. Thus, the further elucidation of the genes and proteins involved in promoting high salt tolerance is important.

Recently, molecular technique has been widely adapted to screen rice varieties to ascertain their potential to grow under stress conditions. On exposure to drought and salinity stress, plants activate or inhibit a series of genes, and the products of these genes may either further control the expression of downstream genes or directly protect plants from stress damage [10]. Salinity tolerance in plants are controlled by a set of inherent multi-genes and established environmental factors, which brings about numerous metabolic changes in each plant part. Salt stress has long been described as a quantitative genetic trait, more studies are being carried out presently on Quantitative Trait Loci (QTLs) linked to salt stress responses in rice, as well as on association studies [11]. The identification of QTLs (Quantitative Trait Loci) has contributed significantly on understanding how traits are being controlled genetically. However, the number of studies of heterogeneous quality conducted on salinity tolerance in rice is still limiting [12]. In rice plant QTLs for salt tolerance have been identified on chromosome 1, housing the major locus Saltol (reported to be involved in Na^+/K^+ homeostasis under salinity) derived from Pokkali and SKC1 (OsHKT1;5) from Nona Bokra [12]. You et al., reported that Transcription Factors (TFs) play significant roles in stress signaling cascades as they are one of the critical regulatory proteins involved in abiotic stress responses, regulating the expression of a class of stress related genes and control of plant resistance to environmental stresses. The potential of rice plant to be susceptible or tolerant to high salinity is associated to the action of multiple stress responsive genes, interacting with other components of stress signal transduction pathways [13]. Waziri et al., asserted that plant breeders have been able to transfer whole saltol QTL that houses the genes working in co-ordination to confer salinity tolerance from tolerant rice varieties to susceptible elite lines using marker.

Most available resources on genetic studies of salinity stress in rice emphasizes on Saltol QTL derived from Indian rice landrace Pokkali which provides seedling stage salt tolerance, with very little work on the genetics of reproductive stage salt tolerance in rice [14]. In a study carried out by Liu et al., five known genes (*OsMYB6*, *OsGAMYB*, *OsHKT1;4*, *OsCTR3*, and *OsSUT1*) and two newly identified genes (*LOC_Os02g49700*, *LOC_Os03g28300*) significantly associated with grain yield and its related traits under saline stress conditions were identified. Suzuki et al., Liu et al., reported that *OsHKT1;4* plays an important role in Na^+ exclusion in stems together with leaf sheaths, thus excluding Na^+ from leaf blades of a japonica rice cultivar in the reproductive growth stage. In a study carried out by Tang et al., *OsMYB6* gene appeared to play significant role as a stress-responsive transcription factor which positively regulates response to drought and salt stress resistance, and concluded that the gene may be used as a candidate gene for molecular breeding of salt-tolerant and drought-tolerant rice varieties. *OsSUT1* is a sucrose transporter gene that plays a role in carbon partitioning, specifically in grain filling and seed germination under salt stress. *OsGAMYB*, a member of the MYB family also plays an important role as a transcription factor positively regulating salinity and drought stress [15].

Therefore, understanding the perceptions of soil salinity, its effects on crop productivity and identifying the varieties that are able to thrive under such condition is important in the development of the best cultivars that addresses the needs of farmers.

This study assessed the growth responses of six (6) rice genotypes under various level of salinity stress using some morpho-physiological parameters to screen and identify salinity tolerant and susceptible rice varieties using pot screening experiment and validated the presence or absence of key genes associated with salt tolerance in rice [16].

Materials and Methods

Experimental site

The experiment was conducted in a screen house at the department of plant biology, faculty of life science, Bayero University Kano, and centre for dry land agriculture, Bayero University Kano.

Samples collection

Four (4) improved rice varieties FARO57, FARO62, FARO44 and FARO67 and two (2) local varieties collected from National Cereals Research Institute Badeggi. Bida, Niger state, and local farmers for the purpose of this study.

An analytical reagent Sodium Chloride (NaCl) was obtained from a chemical store and the department of plant biology laboratory Bayero University Kano for the purpose of this study.

Experimental procedures

The soil used for the experiment was analyzed before the experiment to establish the extent of NaCl concentration in the soil. The soil pH was determined using a pH reader. The electrical conductivity of the soil was determined using an EC meter. Six (6) rice varieties were tested at different NaCl concentrations at the seedling stage to maturity under controlled conditions in the screen house replicated 3 times at the rates of 0, 6, 12 and 14 g per pot to obtain the salinity levels of 0, 4, 8, 10 dS/m, respectively in 5kg soil. The study adopted the procedure described by Senanayakee et al. Seedlings were thinned to three per pot containing a homogeneous mixture of planting medium including soil, farm yard manure. Seedlings were watered with tap water for 21 days. Thereafter, salinity treatment was applied. The control pots were irrigated with tap water throughout the period of the experiment. The treatments including the

control was replicated three times with a spacing of 15 cm and arranged in a completely randomized design.

Genotypes	Institute of origin
FARO 44	NCRI
FARO 57	NCRI
FARO 62	NCRI
FARO 67	NCRI
SUFI	Local farmer
FARAR ZAIRA	Local farmer

Table 1: Genotypes classification and origin.

Data collection

The morpho-physiological characters (number of leaves, plant height, leaf chlorophyll content, shoot fresh and dry weight, root fresh and dry weight, tiller per plant, panicle length, panicle number and 1000 grain weight) were measured at four weeks interval from the first day after salinity application. Plants were then removed from pots and the roots were washed with tap water, the roots and shoots were separated and weighed. All the plant samples (whole plant) were later dried at 70˚C for 48 hours in an oven and dry weight (g plant^{-1}) was determined. IRRI standard protocol Gregorio et al., was used to assess the tolerance of the rice genotypes to salinity conditions.

Plant height

Plant height was measured in cm from the plant base to the tip of the highest leaf using meter rule.

Chlorophyll content

The chlorophyll content was determined using a chlorophyll meter (CCM-200 plus)

Shoot/root fresh weight

The shoot and root fresh weight were determined separately immediately after harvest using a weighing balance, the root was separated from the shoot.

Shoot/root dry weight

The dry weight for shoot and root was determined using a weighing balance after complete drying in an oven for 48 hours.

Panicle length

Panicle length was measured using a meter rule in cm.

Panicle number

Effective tillers of each plant were counted to determine the total number of panicles in each plant.

Number of tillers

Number of effective tillers were recorded through manual counting.

Grain yield per pot

Grain weight per pot was measured after threshing the representative panicle and weighed using a weighing balance.

1000 grain weight

Total grain yield was measured by weighing all the grains harvested from each variety per pot.

Evaluation of salt stress symptoms

Using the modified standard evaluation system (Table 2) in rating the visual symptoms of the injury imposed by the stress, the susceptible genotypes would be discriminated from the tolerant, moderately tolerant and highly tolerant genotypes.

Score	Observation	Tolerance
1	All leaves normal, no leave symptoms	Highly tolerant
3	Nearly normal growth, but leave tips or few leaves whitish and rolled	Tolerant
5	Growth severely retarded; most leaves rolled; only a few are elongating	Moderately tolerant
7	Complete growth cessation; most leaves dry, some plants dying	Susceptible
9	Almost all plants are dead or are dying	Highly susceptible

Table 2: Modified Standard Evaluation System (SES), adapted from IRRI screening rice salinity tolerance.

The differences between genotypes for the recorded characters were tested for significance using Analysis Of Variance (ANOVA). Data collected were subjected to Genstat Statistical software package (Version 17.1.0.13780) for Analysis Of Variance (ANOVA). The means were compared through employing least significance different test at 5% level of significance.

Molecular analysis

Genomic DNA was isolated from leaves of two-week old plants using the modified Cetyl Trimethyl Ammonium Bromide (CTAB) based method of Fulton et al., and amplified using PCR. The purity and concentration of DNA was measured using nanodrop spectrophotometre at the absorption 260/280 nm.

Adopting the method used by Uyoh et-al. The samples were screened using a total of 8 primers designed from the mRNA sequence of the genes in question.

The presence and integrity of the gene was confirmed using PCR analysis on the genomic DNA and gel electrophoresis.

Genes and primer sequences

The genes were searched and identified on NCBI website and their mRna sequences were used to design the primers. The primer sequences are presented in Table 3 below.

Primers	Primer sequences	Product length
MYB6 F1	CGC TGT TTT AGG GTT TGA GA	660
MYB6 R1	GAG CAG CAG ATG TAT CTC CA	
MYB6 F2	AGG CTG TTC CTT CTT GGA CT	395
MYB6 R2	CCA TTT GGC TGA CCT GTG AT	
GAMYB F1	CAG TTG AGA CGC CAT GTA TC	706
GAMYB R1	ATC CCT GTC TGG TTT ACC TG	
GAMYB F2	TGC CCA ATA GCG TTG CTG TA	1210
GAMYB R2	TTC ACG TAG TCC ACC AGG AT	
SUT1 F1	GTT GAT ATG CTT GGC TGT CG	831
SUT1 R1	TGA GTG ACC AGA AGC TGA TC	
SUT1 F2	CAC GTG CTC TAA TGG CTG AT	1520
SUT1 R2	AGA CAG CCT TGA TGC TCT TG	
HKT4 FI	GTT CAC ATC AAT GCT AGG GC	690
HKT4 R1	AAG CTC TCT GAG TTC CAC TC	
HKT4 F2	CATGGC AAC AGT AGA GAT GG	1350
HKT4 R2	GCATCG GAA GGA AGG TAC AT	

Table 3: Primer sequences.

Amplification and agarose gel electrophoresis

Each PCR reaction was carried out with 25.0 µl reaction mixtures containing; 12.5 µl of primer master mix, 1 µl of forward primer, 1 µl of reverse primer, 8.5 µl of nuclease free water and 2.0 µl of each template DNA samples. PCR profile was maintained as initial denaturation at 94˚C for 3 minutes, followed by 33 cycles of denaturation at 94˚C for 30 seconds, annealing at 55˚C-57˚C for 30 seconds, and polymerization at 72˚C for 1 minute; and final extension by 5 minutes at 72˚C. A 1 kb DNA ladder was used to compare the size of the molecule and position of the bands for the sample.

Results and Discussion

Soil and water analysis

The soil and water used for the purpose of this study was analyzed prior to the commencement of the study to establish the level of electrical conductivity in the soil and water. The result is presented in Table 4 below. The chemical and physical properties of the soil and water used for the purpose of this study was analyzed and presented in Table 4. The initial electrical conductivity of the soil was 0.023 dS/m and the pH was 7.8 while that of the water used for irrigation was 7.3 and 0.027 dS/m respectively. All exchangeable cations recorded low values. Similarly, the Exchangeable Acidity (E.A) was low (0.334 cmol/kg) while the cation exchange capacity was (27.165 cmol/kg).The textural class of the soil used for this experiment was a sandy loamy soil.

pH (H_20)	pH ($CaCl_2$)	EC (dS/m)	P (mg/kg)	0.C (%)	N (%)	Ca (cmol/kg)	Mg (cmol/kg)	K (cmol/kg)	Na (cmol/kg)	E.A (cmol/kg)	CEC (cmol/kg)	Sand %	Clay %	Silt %	Textural class
7.88	6.89	0.023	17.116	0.798	0.175	4.565	0.887	0.212	19.138	0.334	27.165	66.64	18.08	15.28	Sandy loam

Table 4: Soil and water analysis result.

Properties of water used for irrigation:

pH-7.3

EC-0.027(dSm)

Classification of genotypes based on IRRI standard evaluation system for rice salinity tolerance: Based on the IRRI score for rice salinity tolerance, the varieties were categorized into four level of stress response presented in Table 5. At 0 dS/m all plant were scored 1 which indicates normal morphological growth and no salt stress symptoms, at 4 dS/m the varieties were scored 3 based on IRRI standard evaluation system, indicating nearly normal growth, but leave tips or few leaves whitish or rolled. At 8 dS/m FARO 44 and 67 were scored 5 indicating retarded growth and most leaves rolling with just few elongated while FARO 62,57, SUFI and FARAR ZAIRA were scored 7 indicating completely cessation of growth with some plants dying. At 10 dS/m FARO 44 and 67 were scored 5 indicating retarded growth and most leaves rolling with just few elongated while FARO 62,57. SUFI and FARAR ZAIRA were scored 9 indicating all plants are dead or dying. On the basis of tolerance and susceptibility indices, FARO 44 and 67 were classified as moderately tolerant varieties, FARO 62 and 57 as susceptible varieties and SUFI and FARAR ZAIRA as highly susceptible varieties.

Concentration					
Genotype	0 dS/m	4 dS/m	8 dS/m	10 dS/m	Tolerance
Faro 62	1	3	7	9	Susceptible
Faro 44	1	3	5	5	Moderately tolerant
Faro 67	1	3	5	5	Moderately tolerant
Faro 57	1	3	7	9	Susceptible
Sufi	1	3	7	9	Highly susceptible
Farar Zaira	1	3	7	9	Highly susceptible

Table 5: Classification of genotypes based on IRRI standard evaluation system for rice salinity tolerance.

Effect of salinity on plant height of some rice (*Oryza sativa* L.) varieties: The main effect of salt concentration and varieties on plant height in rice is presented in Table 6. The result obtained indicated significant differences ($p \leq 0.05$) among the various treatments and genotypes studied at 4 weeks after salinity application. The highest plant height was observed in the control (0 dS/m) plants and the least plant height was recorded in plants treated with 10 dS/m. At 8 weeks after salinity application, plant height was significantly higher ($p \leq 0.05$) in the control plant (0 dS/m), compared to plants treated with 4 dS/m, 8 dS/m and 10 dS/m which recorded lower plant height with 10 dS/m recording the least plant height. Similar trend was observed at 14 weeks after salinity application with plant height being significantly higher in the control plants (0 dS/m) and significantly lower in plants treated with 10 dS/m compared to the control. The effect of variety was also significant ($p \leq 0.05$).

Sources	4 WASA	8 WASA	14 WASA
Treatments			
0 dS/m	51.29^a	69.66^a	116.68^a
4 dS/m	40.69^b	55.95^b	96.73^b
8 dS/m	24.00^c	33.89^c	52.26^c
10 dS/m	11.51^d	14.90^d	20.91^d
LSD	0.901	1.691	2.781
Varieties			
FARO 62	29.11^b	45.46^c	79.68^b
FARO 44	44.04^a	55.50^b	65.48^d
FARO 67	44.76^a	59.46^a	100.91^a
FARO 57	29.98^b	44.19^c	72.93^c
SUFI	21.28^c	36.00^d	54.17^e
FARAR ZAIRA	22.08^c	37.83^d	56.69^e
LSD	1.103	2.07	3.406

Table 6: Effect of different level of salinity on plant height (cm) of some rice varieties (*Oryza sativa* L.).

Tr. * Var	**	**	**
Note: Figures followed by the same letters along the column are not significantly different according to Fisher's protected LSD at 5% level. WASA=Weeks after salinity application. **=Significant at 95% level. LSD: Least Significant Difference; Trt: Treatment; Var: Varieties			

Effect of different level of salinity on chlorophyll content of some rice varieties (*Oryza sativa* L.)

The effect of salinity and varieties on chlorophyll is presented in Table 7. This result indicated that amongst the various salinity treatment levels, the highest concentration of salinity (10 dS/m) recorded the least value of chlorophyll compared with control treatment (0 dS/m). At 8 weeks after salinity application there was significant difference ($P \leq 0.05$) among the various treatments. The highest chlorophyll was observed in control (0 dS/m) and the least chlorophyll was observed in the highest salinity treatment level (10 dS/m). Similarly, at 8 weeks after salinity application there was significant difference in the amount of chlorophyll obtained at the various treatment level, control treatment had the highest chlorophyll (40.67) while the least (8.29) was observed in 10 dS/m. At 14 weeks after salinity application, similar trend was observed with control having the highest chlorophyll content.

The effect of variety indicated that Faro 67 had the highest chlorophyll (16.43) than Faro 44, Faro 57, Faro 62, Sufi and Farar zaira which had the least chlorophyll of (8.70) at 4 weeks after salinity application. At 8 weeks after salinity application, chlorophyll was significantly higher in Faro 44 and Faro 67 with 33.55 and 32.99 respectively and lower in Sufi with 16.14. At 14 weeks after salinity application, Faro 67 and Faro 44 recorded significantly higher chlorophyll ($p \leq 0.05$) than Faro 62, Faro 57, Sufi and Farar Zaira (Table 7).

Sources	4 WASA	8 WASA	14 WASA
Treatments			
0 dS/m	20.31^{a}	40.67^{a}	30.41^{a}
4 dS/m	15.62^{b}	32.67^{b}	26.99^{b}
8 dS/m	8.73^{c}	20.02^{c}	19.87^{c}
10 dS/m	3.76^{d}	8.29^{d}	9.41^{d}
LSD	0.701	1.055	0.872
Varieties			
FARO 62	11.15^{d}	28.61^{b}	24.65^{b}
FARO 44	14.91^{b}	33.55^{a}	29.84^{a}
FARO 67	16.43^{a}	32.99^{a}	29.92^{a}
FARO 57	12.51^{c}	24.79^{c}	23.12^{c}
SUFI	8.95^{e}	16.14^{d}	15.82^{d}
FARAR ZAIRA	8.70^{e}	16.39^{d}	6.67^{e}
LSD	0.858	1.292	1.069
Tr. * Var	**	**	**
Note: Figures followed by the same letters along the columns are not significantly different according to Fisher's protected LSD at 5% level. WASA: Weeks After Salinity Application. **=Significant at 95% level. LSD: Least Significant Difference; Trt: Treatment; Var: varieties			

Table 7: Effect of different level of salinity on chlorophyll content of some rice varieties (*Oryza sativa* L.)

Effect of different levels of salinity on shoot and root fresh and dry weight of some rice varieties (*Oryza sativa* L.)

The main effect of salinity and varieties on shoot fresh weight is presented in Table 8. The result obtained indicated significant differences ($P \leq 0.05$) on shoot fresh weight among the various treatment and genotypes. The result showed that shoot fresh weight was highest in the control 0 dS/m for all varieties and decreased significantly as the concentration of salinity increases, the lowest shoot fresh weight was recorded at 10 dS/m. With respect to the varieties, shoot fresh weight differed considerably, the highest shoot fresh weight was observed in FARO 67, and the least was observed in FARAR ZAIRA.

The result obtained from the estimation of the main effects of salinity and varieties on shoot dry weight is presented in Table 8. The result showed that there was significant ($P \leq 0.05$) difference among the various treatments and genotypes. The highest value (119.33 g) was recorded in the control treatment, while the lowest value (11.47 g) was recorded in the highest salinity level (10 dS/m). This implies that the effect of salinity on shoot dry weight increases with increase in the level of salinity imposed. FARO 67 exhibited the highest shoot dry weight when exposed to salinity stress. The lowest shoot dry weight was recorded in SUFI.

The main effect of salinity and varieties on root fresh weight is presented in Table 8. The result indicated significant ($P \leq 0.05$) difference among the various treatments and varieties. The control plants recorded the highest root fresh weight (167.87 g), while the lowest (20.24 g) root fresh weight was recorded in the highest stress level (10 dS/m). Among the varieties FARO 67 recorded the highest root fresh weight (135.88 g) and the least root fresh weight was recorded in FARO 44 (60.63 g).

Root dry weight: The main effect of salinity and varieties on root dry weight is presented in Table 8. The effect of the treatments on root dry weight was significant ($P \leq 0.05$) and the highest root dry weight (56.34 g) was recorded in the control (0 dS/m), this decreased

across the various treatments with the least root dry weight (7.19 g) recorded in (10 dS/m). FARO 67 was found to have the highest root dry weight (135.88 g) while the lowest root dry weight (23.88 g) was recorded for FARO 44.

Sources	SFW	SDW	RFW	RDW
Treatments				
0 dS/m	339.29^{a}	119.33^{a}	167.87^{a}	56.34^{a}
4 dS/m	241.60^{b}	82.51^{b}	120.02^{b}	42.82^{b}
8 dS/m	129.99^{c}	52.42^{c}	58.97^{c}	19.24^{c}
10 dS/m	36.32^{d}	11.47^{d}	20.24^{d}	7.19^{d}
LSD	15.048	4.497	7.117	2.79
Varieties				
FARO 62	238.19^{b}	104.00^{a}	105.82^{b}	29.39^{c}
FARO 44	116.35^{cd}	33.11^{c}	60.63^{e}	23.88^{d}
FARO 67	286.17^{a}	106.07^{a}	135.88^{a}	40.47^{a}
FARO 57	255.01^{b}	86.95^{b}	95.35^{c}	33.37^{b}
SUFI	122.78^{c}	33.57^{c}	78.52^{d}	31.58^{bc}
FARARZAIRA	102.29^{d}	34.91^{c}	74.46^{d}	29.70^{c}
LSD	18.43	5.508	8.71	3.417
Trt. * Var	**	**	**	**

Note: Figures followed by the same letters along the columns are not significantly different according to Fisher's protected LSD at 5% level. WASA: Weeks After Salinity Application. **= Significant at 95% level. LSD: Least Significant Difference; Trt: Treatment; Var: Varieties; SFW: Shoot Fresh Weight; SDW: Shoot Dry Weight; RFW: Root Fresh Weight; RDW: Root Dry Weight.

Table 8: Effect of different levels of salinity on shoot and root fresh and dry weight of some rice varieties (*Oryza sativa* L.).

Effect of different levels of salinity on yield and yield parameters of some rice varieties (*Oryza sativa* L.)

Number of tillers: The effect of salinity and varieties on number of tillers is presented in Table 9. The data collected showed that there was significant ($P \leq 0.05$) effect on the number of tillers among the various levels of salinity. The highest number of tillers (26.28) was observed in plants at (0 dS/m) and the number decreases as the salinity level increases with the least number of tillers (3.06) observed at (10 dS/m). Among the varieties Faro 44 recorded the highest number of tillers (19.83) while FARAR ZAIRA recorded the lowest number of tillers. The high number of tillers recorded in FARO 44 indicates its tolerance to salinity whereas the low number of tillers in FARAR ZAIRA indicates its susceptibility to salinity stress.

Number of filled panicles: The effect of salinity and varieties on number of panicles is presented in Table 9. The effect of the different levels of salinity and varieties on the number of panicles was significant ($P \leq 0.05$). The number of panicle decreases as the concentration of salinity increases. The highest number of panicles was 21.44 in control treatment and the least was recorded in the highest treatment level (10 dS/m).

Among the varieties, FARO 44 recorded the highest number of filled panicles (17.58) and the least number of panicles was observed in FARAR ZAIRA (9.42).

Panicle length: The effect of treatment and varieties on panicle length is presented in Table 9. With regard to the effect of treatments on panicle length, significant difference was recorded among the various treatments. The highest panicle length (25.32) was recorded in control (0 dS/m) while the lowest length (16.33) was observed in (10 dS/m). In terms of varieties, there was significant difference with regard to panicle length among the various varieties. FARO 67 recorded the highest panicle length (24.23 cm) while the lowest panicle length was recorded in FARAR ZAIRA (17.58 cm).

Grain yield: The effect of salinity and varieties on grain yield is presented in Table 9. The effect of the different level of salinity and varieties on grain yield was significant ($P \leq 0.05$) across the various treatment level. The highest grain yield (69.25 g) was recorded in control (0 dS/m) and this decreases as the salt treatment concentration increases, thereby making the highest salt level (10 dS/m) to have the least grain yield (21.13 g). With regard to varieties, FARO 67 recorded the highest grain yield (57.13 g) while FARAR ZAIRA recorded the least grain yield. The difference in yield among the varieties studied may be as a result of sterility imposed by the salinity stress.

1000 Grain weight: The effect of salinity and varieties on 1000 grain weight is presented in Table 9. The weight of 1000 grain was significant ($P \leq 0.05$) across the different treatments and varieties. The control treatment (0 dS/m) had the highest 1000 grain weight (25.7 g) while the highest stress level (10 dS/m) recorded the lowest (16.96 g) 1000 grain weight. Between the six rice varieties, FARO 67 had the

highest (25.39 g) while FARAR ZAIRA and SUFI had the least weight with (11.01 g and 11.42 g) respectively.

Sources	NOT	NOP	PL	GY	1000 GW
Treatments					
0 dS/m	26.28^{a}	21.44^{a}	25.32^{a}	69.25^{a}	25.7^{a}
4 dS/m	20.06^{b}	16.89^{b}	22.94^{b}	49.73^{b}	22.6^{b}
8 dS/m	10.33^{c}	9.44^{c}	17.59^{c}	30.92^{c}	14.23^{c}
10 dS/m	3.06^{d}	4.22^{d}	16.33^{d}	21.13^{d}	16.96^{d}
LSD	1.406	1.103	0.61	2.622	0.747
Varieties					
FARO 62	15.83^{a}	14.75^{a}	19.10^{b}	41.42^{b}	13.79^{d}
FARO 44	19.83^{a}	17.58^{a}	19.73^{b}	44.30^{b}	23.45^{b}
FARO 67	15.25^{b}	16.58^{b}	24.23^{a}	57.13^{a}	25.39^{a}
FARO 57	18.33^{b}	9.83^{c}	17.68^{c}	42.34^{b}	19.01^{c}
SUFI	10.33^{c}	9.83^{c}	18.47^{d}	28.26^{c}	11.42^{e}
FARARZAIRA	10.00^{c}	9.42^{c}	17.58^{e}	26.11^{c}	11.01^{e}
LSD	1.722	1.351	0.747	3.211	0.915
Trt. * Var	**	**	**	**	**

Note: Figures followed by the same letters along the columns are not significantly different according to Fisher's protected LSD at 5% level. WASA: Weeks After Salinity Application. **=Significant at 95% level. LSD: Least Significant Difference; Trt: Treatment; Var: Varieties; NOT:Number Of Tillers; NOP: Number OF Panicles, PNL: Panicle Length; GY: Grain Yield; 1000GW: One Thousand Grain Weight

Table 9: Effect of different levels of salinity on yield and yield parameters of some rice varieties (*Oryza sativa* L.).

Molecular studies: The results for the molecular studies are presented in the Figures 1-4 below. Four candidate genes (*OsMYB6*, *OsGAMYB*, *OsHKT1;4*, and *OsSUT1*) reported to contribute to salinity tolerance in rice were selected and identified in some of the studied rice varieties. The banding pattern of the genotyes was scored by the presence/absence of bands at the expected position on the gel determined by the molecular ladder.

The outcome of the PCR analysis displayed in the gel electrophoresis for *OsSUT1* gene is presented in Figure 1. The result indicates similar banding pattern in varieties F1, F2 and F3. This indicates the presence of the gene *OsSUT1* in these varieties. Varieties F4, S and FZ displayed no bands and can be inferred from this result that the gene *OsSUT1* is absent in these varieties. Two pairs of primer were used and successfully amplified the gene region with band size (690 bp and 1520 bp).

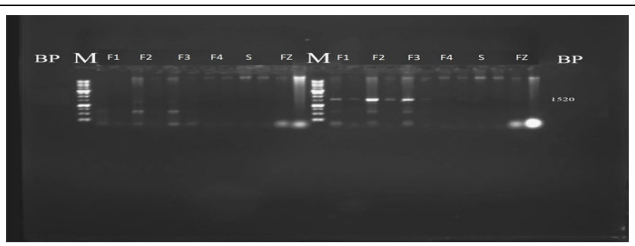

Figure 1: Gel electrophoresis outcome for PCR analysis of 6 rice varieties for the presence of *OsSUT1* gene. The alphabet BP and M indicated in the plate represents (base pairs) and (molecular ladder). F1=Faro 62, F2=Faro 44, F3=Faro 67, F4=Faro 57, S=Sufi, FZ=Farar Zaira.

The outcome of the PCR analysis displayed in the gel electrophoresis for *OsGAMYB* gene is presented in Figure 2. The result indicates similar banding pattern in varieties F2 and F3. This indicates the presence of the gene *OsGAMYB* in these varieties. Varieties F1, F4, S and FZ displayed no bands and can be inferred from this result that the gene *OsGAMYB* is absent in these varieties. Two pairs of primer were used but only one successfully amplified the gene region with band size (1210 bp).

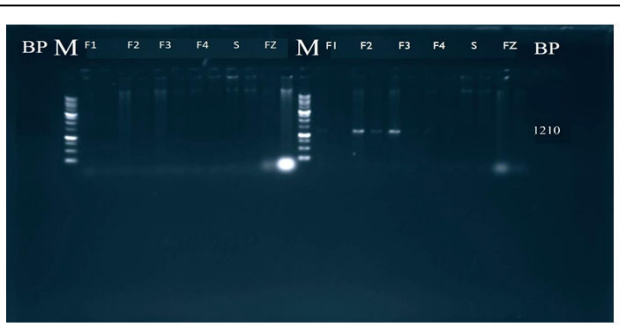

Figure 2: Gel electrophoresis outcome for PCR analysis of 6 rice varieties for the presence of *OsGAMYB* gene. The alphabet BP and M indicated in the plate represents (base pairs) and (molecular ladder). F1=Faro 62, F2=Faro 44, F3=Faro 67, F4=Faro 57, S=Sufi, FZ=Farar Zaira.

The outcome of the PCR analysis displayed in the gel electrophoresis for *OsHKT1;4* is presented in Figure 3. The result indicates similar banding pattern in varieties F1, F2, F3 and F4. This indicates the presence of the gene *OsHKT1;4* in these varieties. Varieties S and FZ displayed no bands and can be inferred from this result that the gene *OsHKT1;4* is absent in these varieties.

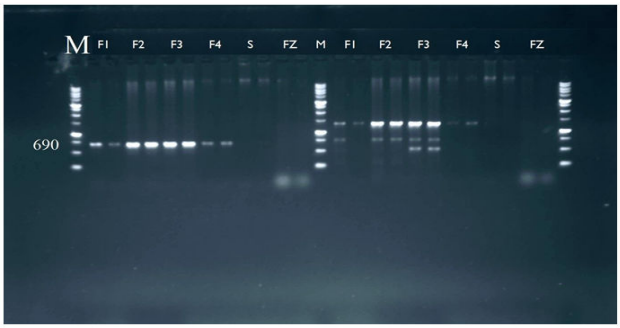

Figure 3: Gel electrophoresis outcome for PCR analysis of 6 rice varieties for the presence of *OsHKT1;4* gene. The alphabet BP and M indicated in the plate represents (base pairs) and (molecular ladder). F1=Faro 62, F2=Faro 44, F3=Faro 67, F4=Faro 57, S=Sufi, FZ=Farar Zaira.

Two pairs of primer were used and both successfully amplified the gene region with band size (690 bp and 1350 bp).

The outcome of the pcr analysis displayed in the gel electrophoresis for *OsMYB6* gene is presented in (Figure 4). The result indicates similar banding pattern in varieties F1, F2, F3, and F4. This indicates the presence of the gene *OsMYB6* in these varieties. Varieties S and FZ displayed no bands and can be inferred from this result that the gene *OsMYB6* is absent in these varieties.

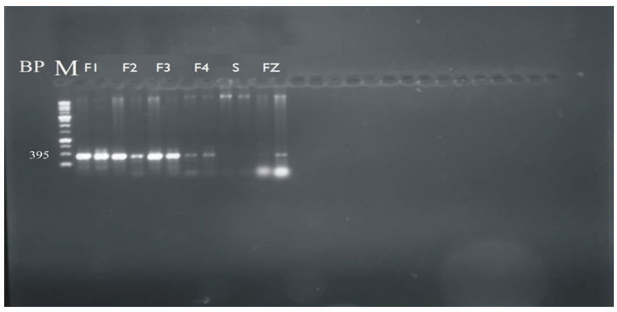

Figure 4: Gel electrophoresis outcome for PCR analysis of 6 rice varieties for the presence of *OsMYB6* gene. The alphabet BP and M indicated in the plate represents (base Pairs) and (molecular ladder). F1=Faro 62, F2=Faro 44, F3=Faro 67, F4=Faro 57, S= Sufi, FZ=Farar Zaira.

Gene enrichment analysis: To determine the functions of the candidate genes, GO enrichment analysis carried out they regulate. The analysis result presented in Table 10 was obtained by pasting the gene ID's on GO analysis website and the result revealed the type of function, position and description of the genes. The result shows that GAMYB a protein coding gene is located on chromosome 1 and functions as transcriptional activator of gibberellin-dependent alpha-amylase expression. *OsSUT1* which is mapped on chromosome 3 functions as sucrose transporters. The result also revealed that OsHKT4 a protein coding gene which is positioned on chromosome 4 functions as ion transporters. The analysis result indicated that LOC_0S04G58020.1 representing *OsMYB6* is not mapped, hence the description and position of the gene wasn't obtained.

To learn about the function of the studied candidate genes, GO enrichment analysis was performed on the four candidate genes. Three of these genes were well represented in the three GO classes of biological process, cellular component and molecular function. The most commonly used GO terms for these classes is presented in Figures 5-8. The most common GO terms in the biological process category were the maltose transport, sucrose transport and pollen germination. The specific GO terms in the cellular component indicate that the genes are "integral component of the plasma membrane and intrinsic component of the plasma membrane". The most common GO terms in the molecular function were maltose transmembrane transporter activity, sucrose proton symporter activity and organic hydroxyl compound transmembrane transporter activity.

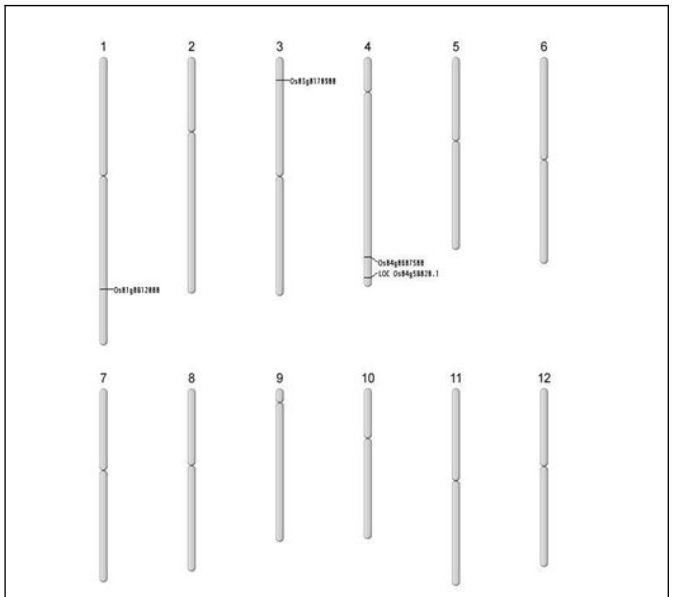

Figure 5: Map of the 12 rice chromosomes showing the position of the salt-responsive biosynthesis related genes.

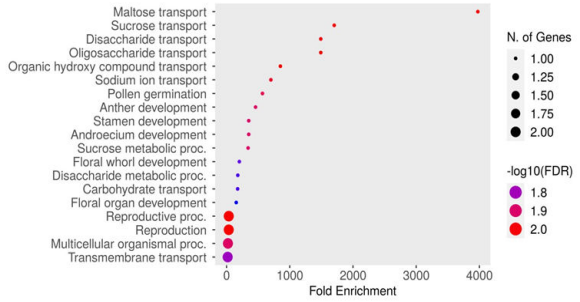

Figure 6: Go enrichment analysis result for biological process.

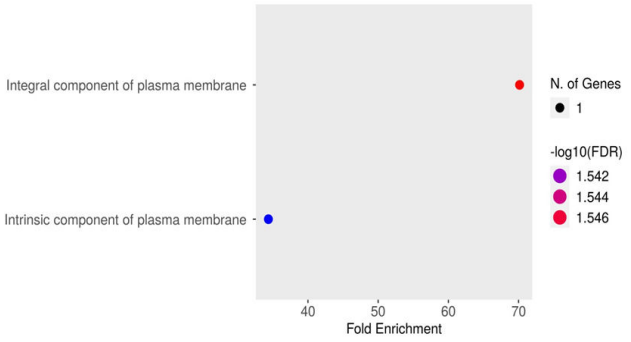

Figure 7: Go enrichment analysis result for biological process.

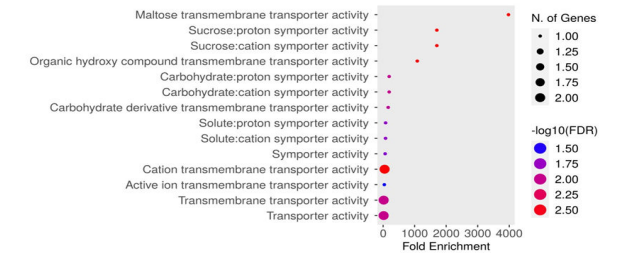

Figure 8: Go enrichment analysis result for molecular function.

The result for the enrichment analysis revealed that the specific biological function for *OsMYB6* gene is not yet clear. In this study the 6 rice genotypes were accessed to determine if this gene is present and two (4) varieties (Faro 62, 44, 67 and 57) which happened to be improved variety appeared to have this gene while the gene was absent.

The result obtained from the gene enrichment analysis indicates that *OsGAMYB* gene which is mapped in chromosome (1) functions as transcriptional activator of gibberellin-dependent Alpha-amylase in rice plant. The GO analysis revealed that the gene is enriched during another development and reproductive pathways of rice plant under salt stress condition. The enrichment result described *OsSUT1* gene as sucrose transporter mapped on chromosome (3). The result showed that this gene was mainly enriched in maltose and sucrose transport. *OsHKT4* gene was described as ion transporter and Na^+ transporter by the gene enrichment analysis and is mapped on chromosome (4). The analysis revealed that sodium ion transport and cation transmembrane transporter activity were enriched by this gene.

In addition to GO, a hierarchical clustering tree summarizing the correlation among significant pathways listed in the enrichment tab (Figure 9). Pathways with many shared genes are clustered together.

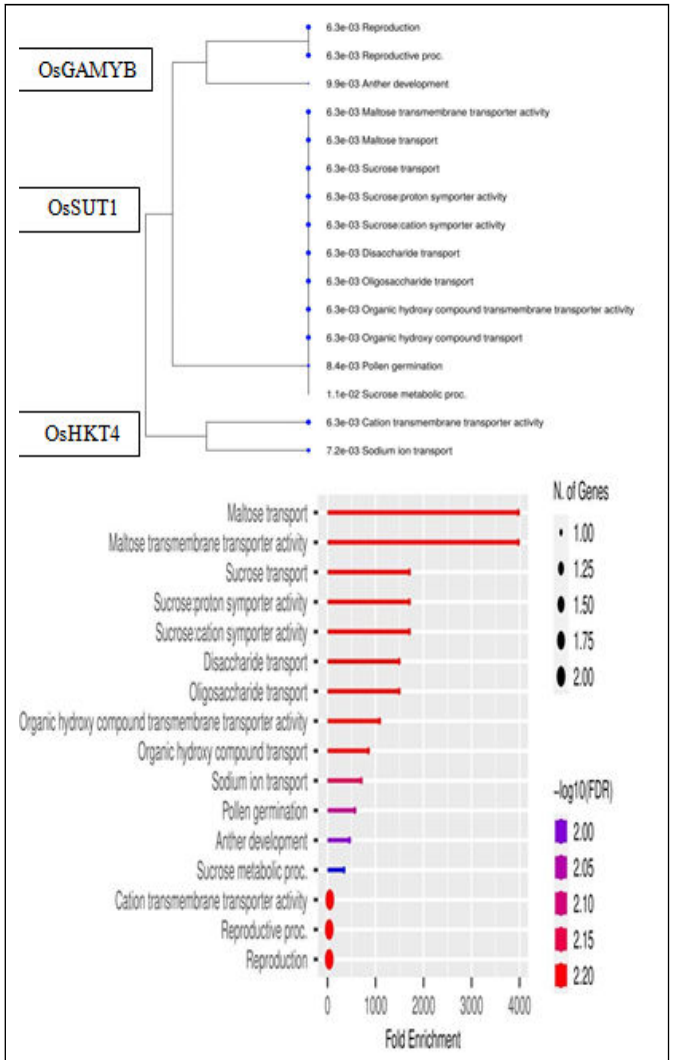

Figure 9: A Hierarchical clustering tree summarizing the correlation among significant pathways listed in the enrichment tab.

Pathways with many shared genes are clustered together. Bigger dots indicates more significant P-values.

Bigger dots indicates more significant P-values. Cluster GAMYB suggests that the gene regulates reproduction, and anther development pathway. Cluster OsSUT1comprising of a large group of pathway suggests that the gene regulates; maltose transport, sucrose transport, disaccharide transport, oligosaccharide transport, organic hydroxy compound transport, and pollen germination. The last cluster group OsHKT4 indicates that the gene regulates cation transmembrane transporter activity and sodium ion transport. The enriched pathways by the genes were associated with interacting networks, showing a close relationship in terms of phenotypic regulation (Figure 10). Pathways connected by the nodes represented those with key regulatory roles.

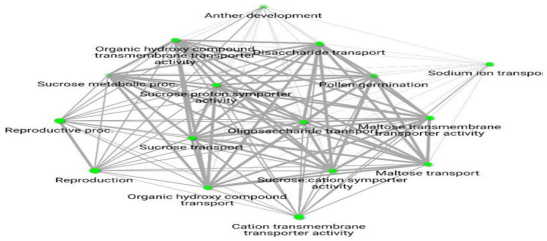

Figure 10: Interactive plot showing the relationship between enriched pathways. Two pathways (nodes) are connected if they share 20% (default) or more genes.

Ensembl					
	Symbol	**Gene ID**	**Type**	**Chr**	**Description**
812000	GAMYB	OS01G0812000	protein_coding	1	Transcriptional activator of gibberellin-dependent Alpha-amylase expression.
170900	OsSUT1	OS03G0170900	protein_coding	3	Sucrose transporter
607500	HKT4	OS04G0607500	protein_coding	4	Ion transporter, Na^+ transporter
S04G58020.1	NA	Not mapped	NA	NA	NA

Table 10: Gene enrichment analysis result.

Discussion

Standard evaluation score of visual salt injury is a widely used screening technique for salinity tolerance in rice. In the present study, the rice accessions were classified as moderately tolerant (FARO44 and FARO 67), susceptible (FARO 62 and FARO 57) and highly susceptible (SUFI and FARAR ZAIRA). The effect of the different level of salinity significantly affected plant height of all varieties, the effect increased as the concentration of the stress level increased. For all varieties, plant height was highest in the control. Plant height significantly reduced with each salt concentration and time of imposition and when concentration was increased to 8 dS/m and 10 dS/m for 8 and 14 weeks, the stress resulted in plant death. Similar observations in several studies on rice varieties have been reported. Puvanitha et al., witnessed that plants height in rice cultivars reduced significantly when exposed to salt stress compared to the control. Efisue et al., also reported that at 6 dS/m the effect of salinity was high and resulted in the decrease of plant height and other growth parameters in rice. This could be as a result of the disturbance of metabolic processes imposed when osmotic stress reduces water uptake by the roots. Generally salt stress affects crops by osmotic, specific ion, ion imbalance and oxidative damage. Excess Na^+ in plants harms cell membrane and organelles of plants and ultimately results in reduction in plant physiological processes leading to plant cell death. On the basis of tolerance and susceptibility two varieties; FARO 44 and FARO 67 demonstrated some level of salt tolerance by maintaining consistence growth under increasing salt tolerance by maintaining consistence growth under increasing salt tolerance by maintaining consistence growth under increasing salt concentration. However varieties; FARO 62, FARO 57, SUFI and FARAR ZAIRA demonstrated constant decrease or cessation of growth under salt stress, indicating some level of susceptibility.

The amount of chlorophyll in the varieties decreased significantly with increase in salt stress concentration. However the plants showed almost uniform green color in the non-stressed condition (control). This decrease could be due to the fact that the stress imposed caused a decrease in the synthesis of the chlorophyll pigment and also caused a high rate of chlorophyll degradation susceptible genotypes exhibited varied symptoms like yellowing of leaves and ultimate death of seedlings at early growth stage. Similar result was reported by Ikhajiagbe et al., who discovered that the leaves of rice plants under stressed conditions exhibited a yellowish white, lethal color, indicating the presence of carotenoid pigment and absence of chlorophyll. Reduced chlorophyll and photosynthetic enzymes like rubisco are the most affected processes of photosynthesis in plants growing under saline condition. It has been observed that plants have the photosynthetic capacity to adjust and match the conditions they are grown under. However, not all plants have this capacity. In this study chlorophyll was measured at 4, 8, and 14 weeks after salinity application at the different salinity level (0, 4, 8 and 10 dS/m). Varieties; FARO 44 and 67 appeared to have the highest chlorophyll content at 4, 8, and 14 weeks after salinity application, this may be because they were able to match/adapt to the stress condition thereby

preventing a high rate of chlorophyll degradation and a decrease in the synthesis of chlorophyll pigment. The least chlorophyll was recorded in SUFI and FARAR ZAIRA, these varieties appeared to be mostly affected by the different stress levels imposed. Therefore the low chlorophyll in these varieties was attributed to a high rate of chlorophyll degradation, reduced photosynthetic enzymes and low potential for the synthesis of chlorophyll pigment caused by the high amount of Na^+ and Cl^{-}. This is in agreement with the findings of Hussain et al., who reported that chlorophyll contents in rice leaves are damaged by the addition of Na^+ and Cl^-, which might hinder the major electron transport in PSII.

Ashrafuzzaman, et al., also reported that stress significantly reduced the content of total chlorophyll in the leaf and this is attributed to the destruction of chlorophyll a, which is considered to be more sensitive than chlorophyll b. Heidary also reported that by increasing salinity levels, chlorophyll a and b decreases and this could be as a result of photo inhibition or decrease in chlorophyll content.

The effect of the different level of salinity significantly affected number of tillers per plant, the effect increased with increase in the concentration of the salt stress. Tiller number was highest in the control plants, however as the concentration of the stress increased, the number of tillers decreased at 4, 8 and 10 dS/m throughout the period of the experiment. The decrease in tillering capacity might be due to the toxic effect of salt on plant growth. This conforms to the findings of Negrao, et al., who reported that high Na^+ concentration affects the tillering ability in rice. Similar finding was reported by Umego, et al., who also observed a significant decrease in tiller number as salt stressed level increased. Zeng and Shannnon also reported that tiller number per plant was significantly reduced at 4.5 dS m and higher salinity level. Tiller number is an important parameter under salt stress that determines the number of grain bearing panicles in rice plant. FARO 44 demonstrated some level of salt tolerance by having the highest number of tillers under increasing salt tolerance. However varieties; FARO 57, FARO67, FARO 62, SUFI and FARAR ZAIRA demonstrated deceased number of tillers or cessation of growth under salt stress, indicating some level of susceptibility.

Panicle number is considered as the main factor affecting rice yield. Salinity decreases yield through decreasing the number of filled panicles. In this study, the number of panicles was highest in the control. Panicle number significantly decreased with increase in salt concentration. The least number of panicle was recorded at the highest stress level 10 dS/m. The decrease in the number of panicle was attributed to the disturbance of metabolic processes and the effect of the toxic ions on the susceptible varieties, resulting in the complete cessation of growth and death of some plants therefore making it difficult for the plants to reach the reproduction stage. This is in agreement with the finding of Negrao, et al., who reported that severe stress level (NaCl > 100 mM), results in the death of rice plant before maturity, making it impossible for susceptible varieties to reach reproduction stage. Similar finding was reported by Ikhajiagbe and Omoregie who disclosed that the number of panicles in their study was reduced by salt stress level for all varieties. Among the varieties, FARO 44 had the highest number of panicle while FARAR ZAIRA had the least number of panicles. The high number of panicle in FARO 44 was attributed to its resilience under the stress condition.

From the result, the values obtained for shoot fresh weight was significantly higher in the control than those at the different salinity levels (4, 8, and 10 dS/m). Shoot fresh and dry weight decreased with increase in the level of salt stressed imposed and drastic reduction was found at the highest stress level 10 dS/m .Varieties differ in the response of shoot fresh weight to salt, FARO 67 had the highest shoot fresh and dry weight while FARAR ZAIRA had the lowest shoot fresh and dry weight. This decrease in the weight could be because of the osmotic stress imposed by high Nacl concentration which made it difficult for substrate or solute to be moved to the various part of the plant from the soil, ultimately resulting in limited plant growth and crop productivity. This is in agreement with the finding of Haq, et al., who reported significant reduction in shoot fresh and dry weight with increase in salt level and attributed this to the decrease in water potential of rooting medium and growth decline related to osmotic effect under salt stress. Similar result was also reported by Puvanitha, et al., who disclosed that salinity significantly reduced shoot dry weight and this may be due to the toxic effects of Nacl and unbalanced nutrient uptake by the plant.

The roots are in direct contact with the surrounding solution. Hence they are the first to encounter the effect of the stress and as a result develop tolerance or get damaged by the salt stress imposed. Root fresh and dry weights were significantly affected by the different level of salinity. The control plants had the highest root fresh and dry weight and this significantly decreased with each salt concentration from the period of stress imposition to when the experiment was concluded. The reduction of the plant root weight may be due to the osmotic and ionic effect of Nacl imposed on the plants. This result is similar to the finding of Puvanitha et al., who reported that reduction in root weight due to increased salinity may be a result of a combination of osmotic and specific ion effects of Cl and Na^+. Rahman, et al., also reported that salt stress (100 mM NaCl 10 dS/m) reduced root length, root dry weight, shoot length and shoot dry weight both in sensitive and in tolerant cultivar where growth reduction is higher in sensitive cultivar compared with tolerant varieties. Among the varieties, FARO 67 had the highest number of root fresh and dry weight while FARAR ZAIRA had the root fresh and dry weight.

Panicle length was significantly affected by the different level of salt stress in the selected genotypes. The control plants had the highest panicle length while the least panicle length was observed at 10 dS/m. This indicates that increase in the level of salt stress imposed caused a further reduction in the length of panicle. The reduction in panicle length might be as a result of the disruption of the metabolic process in the plant due to the effect of the stress. This result agrees with the finding of Auyo, et al., who reported that there was a significant difference among the varieties in respect to panicle length and this might be because of the negative effects of the induced salinity during seedling growth. Among the varieties, FARO 67 and 44 had the highest panicle length, while FARAR ZAIRA had the least panicle length. Similar finding was made by Kranto, et al., who reported a decrease in the mean values of panicle length, plant height, shoot fresh weight, and K^+ for all varieties studied at the different stress level induced.

The findings of this study revealed that the effect of the different salt level of salt stress imposed had significant effect on rice grain yield and 1000 grain weight. The study indicates that rice yield decreases with increase in the induced salinity level, the highest grain yield and 1000 grain weight was observed in the control plants while the least grain yield and 1000 grain weight was recorded at 10 dS/m. This finding is in accordance with that of Aref et al. The reduction in yield might be due to the alteration of various metabolic processes in plants under stress. Ashrafuzzaman, et al., reported that grains act as a physiological sink in grain filling process, and the leaves servers as the source. The effect of salinity enhances senescence thereby hampering the grain filling process and as a result sterility is induced and grain size is hindered. Among the varieties, FARO 67 had the highest yield and 1000 grain weight with while FARAR ZAIRA had the lowest yield and 1000 grain weight.

At present, many genes related to rice salinity tolerance have been identified by various methods. These genes are reported to be potential candidates to increase salinity tolerance in rice. The presence/absence of *OsMYB6* gene in the six rice varieties was determined. The gel electrophoresis result indicated the presence of this gene in FARO 44, 67, 57 and 62. Genes encoding MYB-type transcription factors have been reported to play important role in abiotic stresses in many plant species. Tang, et al., reported that *OsMYB6* gene appeared to play a significant role as a stress responsive transcription factor which positively initiates and regulates response to drought and salt stress. The level of expression of this gene regulates important pathways involved in the stress tolerance. Liu et al.; Tang et al.; reported that overexpression of *OsMYB6* increases tolerance in rice plant. Hence we assumed that the gene was most likely more expressed in FARO 44 and 67 because they appeared to show some level of tolerance to the stress compared to other varieties; Faro 62, 57, SUFI and FARAR ZAIRA that appeared susceptible with respect to the morphological data collected. The outcome of GO enrichment analysis described OsHKT4 as a protein coding gene which functions as ion transporters *OsHKT4* gene belongs to the high affinity K^+ transporter (HKT) family. The HKT family is active at the plasma membrane and permeable to either K^+ and Na^+ or to Na^+ only and plays an important role in plant salt tolerance or growth in conditions of K^+ shortage. The gene has been reported by several authors to contribute towards the exclusion of Na^+ in leaves and stems of rice plant under salt stress conditions. Suzuki, et al., reported that the gene functions in the exclusion of Na^+ in stems and leaf sheath of rice plant. Lui, et al., also reported that this gene mediates the exclusion of Na^+ from rice leaf blades during salt stress *via* Na^+ selective transport. From the gel electrophoresis result, the presence of this gene was detected in FARO 62, 57, 44 and 67 and was not detected in varieties SUFI and FARAR ZAIRA. The presence of this gene and level of expression might be attributed to the outstanding performance of FARO 44 and 67 under the different salt levels induced in this study. One of the toxic effects of salt stress is the high imposition of Na^+. The ability of this gene to exclude Na^+ from the leaves and stem of rice plants may have also have contributed to the reason why FARO 44 and 67 performed better than other varieties with respect to the morphological and physiological data collected. *OsSUT1* gene was described as sucrose transporter by the enrichment analysis mainly enriched in maltose and sucrose transport. It is the major salt responsive gene of the 5 *OsSUT*-genes that functions in carbon partitioning specifically for grain filling and seed germination. The gene comprises of a large group of pathway regulating; maltose transport, sucrose transport, disaccharide transport, oligosaccharide transport, organic hydroxy compound transport, and pollen germination. The presence of this gene was detected in FARO 62, FARO 44 and FARO 67.

Thus the study assumed the gene to have contributed to their salt acclimation response. The level of expression of this gene determines how the varieties respond under the salt stress condition. Siahpoosh, et al., reported that the levels of expression of this gene were found to be down regulated after long exposure to saline soil in salt sensitive varieties. The gene presence wasn't detected in FARO 57, SUFI, and FARAR ZAIRA and we attributed the absence of this gene to the lack of protein encoding sequence for this gene in these varieties. OsGAMYB a protein coding gene located on chromosome 1 functions in the induction of gibberellin-dependent alpha-amylase in the aleurone and floral organ and pollen development in rice. The gene functions in parallel with UDT1 to regulate early anther development. Wang, et al., reported that this gene regulates and increases tiller number and grain yield under stress condition particularly salinity and drought stress. We believed the presence of this gene in (FARO 44 and FARO 67) increased their yield and tiller number under the stress condition because the gene is enriched during another development and reproductive pathways of rice plant under salt stress condition.

Conclusion

In this study, we understood that NaCl stress reduced plant height, chlorophyll content, biomass, dry matter, yield and yield parameters in all the six rice varieties, however Faro 44 and 67 exhibited better performance with regards to the parameters measured and appeared to be the varieties that survived under the highest salinity level (10 dSm). The result for the morphological study was further authenticated by carrying out molecular studies, the result obtained revealed the presence of some selected candidate genes reported to be associated with salinity stress tolerance in rice, thus demonstrating the high genetic potential for salinity tolerance in this varieties (Faro 44 and 67). Furthermore, the results also revealed that (Faro 62, 57, Sufi and Farar zaira) were significantly affected by the induced salt stress and at the highest stress level, growth was completely seized in these varieties and death ultimately ensued. The molecular analysis revealed the absence of the selected candidate genes in these varieties; hence we classified them as susceptible varieties. However, further research should be conducted under field conditions in order to authenticate these findings. FARO 44 and FARO 67 which performed better under the highest stress level should be subjected to further trial to establish a better level of performance at the studied concentration. More physiological features should be assessed to understand better the effect of toxic ions imposed by salinity stress on the physiology of these plants. The level of expression of the selected candidate genes should be assessed using (RT-PCR) to quantify the contribution of the genes towards tolerance to salt stress. The studied candidate genes should be cloned in varieties that appeared to lack them.

Acknowledgements

I wish to acknowledge with profound gratitude the effort of my supervisor as well my mentor Assoc. Prof. Lawan Abdu Sani, whose dedication, commitment and effort were imperative and resourceful for the success of my academics. I also wish to express my sincere appreciation to Dr. Babura, Mallam Hassan Khalid and my colleagues, whose advice and g uidance were much enjoyed. Finally, I acknowledge

with utmost appreciation the staffs of plant biology department, agronomy department, and the centre for dry land agriculture for their assistance in seeing me conduct this research work successfully.

References

1. Aref F (2013) Effect of saline irrigation water on yield and yield components of rice (*Oryza sativa* L.). Afr J Biotechnol 12.
2. Ashrafuzzaman M, Khan MA, Shohidullah SM, Rahman MS (2000) Effect of salinity on the chlorophyll content, yield and yield components of QPM (Quality Protein Maize) cv. Nutricta. Pak J Biol Sci 3.
3. Auyo MI, Aliyu A, Zakari MM (2022) Effects of salinity on yield and yield components of some varieties of rice (*Oryza sativa* L.). J Sci Tec Educ 9: 349-357.
4. Chang KK, Hyeon SJ, Hak BK, Doh WY, Gang SL, et al. (2013) Identification of salt-responsive biosynthesis genes in rice *via* microarray analysis. J Rice Res 1: 1-6.
5. Almeida DM, Almadanim MC, Lourenço T, Abreu IA, Saibo NJ, et al. (2016) Screening for abiotic stress tolerance in rice: Salt, cold, and drought. Methods Mol Biol 155-182.
6. Food and Agricultural Organisation (2008) FAO Land and plant nutrition management service.
7. Gregorio GB, Senadhira D, Mendoza, RD (1997) Screening rice for salinity tolerance.
8. Hussain S, Zhang JH, Zhong C, Zhu LF, Cao XC, et al. (2017) Effects of salt stress on rice growth, development characteristics, and the regulating ways: A review. J Integr Agric 16: 2357-2374.
9. Ikhajiagbe B, Omoregie UE (2020) Growth, yield, genetic parameters and Random Amplified Polymorphic DNA (RAPD) of five rice varieties treated with sodium azide and sown under different saline conditions. Bull Natl Res Cent 44: 1-9.
10. James SD (2018) Effects of salinity on growth and yield of rice (*Oryza sativa* L.) and development of tolerant genotypes in Kilosa district, Tanzania. Sokoine university of agriculture Morogoro, Tanzania
11. Jamil M, Charnikhova T, Houshyani B, van Ast A, Bouwmeester HJ (2012) Genetic variation in strigolactone production and tillering in rice and its effect on Striga hermonthica infection. Planta 235: 473-484.
12. Kargbo SS, Showemimo FA, Porbeni JB, Akintokun PO (2019) Response of rice genotypes to salinity under hydroponic conditions. Agro Sci 18: 11-18.
13. Shrivastava P, Kumar R (2015) Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. Saudi J Biol Sci 22: 123-131.
14. Liu C, Chen K, Zhao X, Wang X, Shen C, et al. (2019) Identification of genes for salt tolerance and yield-related traits in rice plants grown hydroponically and under saline field conditions by genome-wide association study. Rice 12: 1-3.
15. Liu G, Li X, Jin S, Liu X, Zhu L, et al. (2014) Overexpression of rice NAC gene *SNAC1* improves drought and salt tolerance by enhancing root development and reducing transpiration rate in transgenic cotton. PLoS One 9: e86895.
16. Negrao S, Courtois B, Ahmadi N, Abreu I, Saibo N, et al. (2011) Recent updates on salinity stress in rice: From physiological to molecular responses. Crit Rev Plant Sci 30: 329-377.