



Salinity stress effects on some morpho-physiological traits of selected rice (*oryza sativa* L.) genotypes

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Abstract

A study was conducted to evaluate the responses of selected rice genotypes at various levels of salinity to identify susceptible and tolerant parents for breeding purpose. The evaluation was done at the seedling stage at the Department of Crop Sciences and Horticulture of the Sokoine University of Agriculture, during November and December 2015. The eight rice genotypes were evaluated at three NaCl concentrations (0 mMNaCl, 50 mMNaCl and 100 mMNaCl). Salt injury was scored on a 1-9 scale based on seedling growth characteristics following the modified Standard Evaluation Score (SES) of the International Rice Research Institute. The percent relative reduction (% RR), salinity tolerance index (STI) and salinity susceptibility index (SSI) were used to rank genotypes as tolerant or susceptible. On the basis of SES, phenotypic observation, the three indices and dry matter (DM) reduction, three rice genotypes (FL 478, IRRI 128, IR65192-4B-20-3,) were identified as salt tolerant; IRRI 113 and IRRI 112 were moderately tolerant while Suakoko-10, NERICA-L-19 and IRRI 124 were identified as salinity susceptible genotypes. Therefore, Fl 478, IR65192-4B-10-3 were selected as donor parents; similarly, SUAKOKO-10 and NERICA-L-19 were selected as recurrent parents to be used in a breeding program.

Keywords: Salinity Stress, Genotypes, Indices, Tolerance, Susceptible, Homogeneous Mixture

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1. Introduction

There is wide range of variations among the cereals for salt tolerance, and rice has shown to be the most sensitive cereal to salinity and barley the most tolerant cereal (Munns and Tester, 2008; and Karan et al., 2012). The production of crop worldwide is severely affected by presence of high salinity (sodium chloride) in soil and irrigation water (Maibody and Feizi, 2005; Demiral and Turkan, 2005). Globally, rice is one of the most important crops, but it is seriously affected by soil salinity. Rice responds to salt stress in the same manner as other glycophytes by using a number of strategies which include minimizing influx, maintaining efflux, and translocation and compartmentalizing potentially toxic ions such as Na^+ and Cl^- (Tester and Davenport, 2003; Kader et al., 2006; Anil et al., 2007).

Soil salinization has become one of the major environmental problems affecting plant growth and productivity worldwide (Allakhverdiev et al., 2000). Salinity affects plants by inducing water deficit in plants even in well watered soils by decreasing the osmotic potential of soil solutes which makes it difficult for roots to take up water from the soil (Sairam et al., 2002). Salinity can affect crop by either causing the death of the crop or decreasing the productivity of the crop (Parida et al., 2004).

Salt stress can lead to a considerable decrease in the fresh and dry weights of leaves, stems, tillers, fertile tillers and roots of susceptible genotypes (Chartzoulakis and Klapaki, 2000). In susceptible plants, high ionic concentration in soil competes with the uptake of essential nutrients, especially K^+ , leading to K^+ deficiency. The treatment of soil with NaCl increases the concentration of Na^+ and Cl^- level in soil and subsequently increases their uptake by susceptible plants; thus, high concentration of Na^+ and Cl^- in plant affects the uptake of Ca^{2+} , Mg^{2+} and K^+ by the plants (Khan et al., 1999). Other Natural boundaries imposed by soil salinity are the limiting of caloric and the nutritional potential of agricultural production (Keshtehgar et al., 2013). Therefore, the objective of this study was to identify genotypes to be used as recurrent and donor parents in a breeding program.

2. Materials and methods

Eight rice genotypes, six from the International Rice Research Institute (IRRI) and two from the AfricaRice Center, were tested at different NaCl concentrations at the seedling stage under controlled condition in a screen house at the Sokoine University of Agriculture (SUA) in 2015 (Table 1). IRRI standard protocol (Gregorio et al., 1997) was used to evaluate salt tolerance of rice genotypes. The rice genotypes were grown under three concentrations of salinity stress namely 100 mMNaCl , 50 mMNaCl and 0 mMNaCl using a randomized complete block design arranged in factorial with 3 replications. Prior to planting, seeds were germinated in glass petri-dishes and three seedlings transplanted per pot (with dimension, 18cm x 19cm) containing 1.7 kg of homogeneous mixture of planting medium including soil, farm yard manure and rice husk in the ratio 6:2:10. Seedlings were watered with distilled water for 21 days after transplanting then salinity treatments were applied 21 days after transplanting and continued once every week until the end of data collection. The control pots were irrigated with distilled water once weekly until the end of data

collection, which was done 22 days after the salinity treatments were applied. Two hundred milliliters of NaCl solution was applied twice a week to each treatment pot.

Table 1. Modified standard evaluation score (SES) of visual salt injury at seedling stage

Scores	Observation	Tolerance
1	Normal growth on leaf symptoms	Highly tolerant
3	Nearly normal growth, but leaf tips or few leaves whitish and rolled	Tolerant
5	Growth severely retarded; most leaves rolled; only a few are elongating	Moderately tolerant
7	Complete cessation of growth; most leaves dry; some plants dying	Susceptible
9	Almost all plants dead or dying	Highly susceptible

Source: Gregorio et al. (1997), IRRI

Plant height was measured from the base of the plant (top of the soil) to the tip of the tallest leaf after 22 days of salinity application. Plants were then removed from pots 22 days after the application of salinity stress; the roots of each plant were washed with tap water and rinsed with distilled water and then blotted dried using blotting paper and the roots and shoots were separated. All the plant samples (whole plant) were dried at 70 °C for 48 hours in an oven to a constant weight and dry weight (g plant⁻¹) was determined. After dried shoot and root were weighed on an electronic beam balance and ground to powder, Na, Mg²⁺, Ca²⁺, K⁺ and K⁺/Na⁺ were determined using the ashing method at a temperature of 550 °C to 600 °C. Na⁺ and K⁺ contents (Cmol kg⁻¹ dry weight) of shoots and roots were determined from a 0.5g dried digested sample using a flame photometer. Ca²⁺ and Mg²⁺ content of shoot and root determined from 0.5g to 1g in a crucible using a muffle furnace heated at 550 °C to 600 °C for 2 hours, and final reading done from an atomic adsorption spectro-photometer.

The percent relative reduction (RR %) of morphological traits was calculated as: [RR% = 1-(biomass under salinity/biomass under control), (Mohammad et al., 2014)]. The Salinity susceptibility index (SSI) was determined as,

$$SSI = \frac{YW - YD}{SII(YW)}$$

where YW and YD are the mean biomass of a given accession in saline and non-saline conditions respectively, and SII was the salinity intensity index, calculated as

$$SII = \frac{1 - XS}{XN}$$

where: XS and XN, are the means of all accessions under salinity stressed and non - stressed environments respectively (Farid and Ali, 2012). The SSI as an index provides an assessment of the relative performance of a given entry with regard to the mean performance of all the genotypes Fischer & Maurer (1978). Salinity

tolerance index (STI) was calculated as total dry weight of plant obtained from different salt treatments concentrations compared to total plant dry weight obtained from control.

$$STI = \frac{TDW_{sx}}{TDW_{si}} \times 100$$

where; TDW=total dry weight, Si =control treatment, Sx= salt level treatment (Seydi, 2003).

The indices were used to rank the rice genotypes in terms of tolerance to high concentration of NaCl (100mMNaCl). Salinity Data obtained were subjected to analysis of variance using the Genstat Statistical Package 14th edition (Goedhart and Thissen, 2011). Treatment means were compared using Tukey Honestly Significant Test (HSD).

The soil used for the experiment was analyzed before and after the experiment to establish the extent of NaCl concentration in soil as result of irrigation. Soil was sampled at the crop museum (a site for field practical) at SUA at a depth of 30 cm and air dried, sieved through 2 mm mesh and then the pH, Ece, Na, K, Ca and Mg contents were determined. Soil pH was determined using the pH reader (Hanna Instrument pH Meter, Model Hi 9032) in a 1: 2.5 soil water ratio. Electrical conductivity was determined by the portable electrical conductivity meter (Hanna Instrument Conductivity Meter, Model Hi 9032) in 1:2.5 soil water ratios (Jackson, 1973). Available potassium and sodium, Magnesium and Calcium were determined using 1N NH₄OAc at pH7 and followed by quantification using Atomic Adsorption Spectro-photometer.

The exchangeable sodium percentage (ESP) was determined using the following equation:

$$ESP = 0.94 + 1.119SAR$$

CEC is WAS estimated as the sum of the major exchangeable cations, including hydrogen. While SAR was determined using the following equation:

$$SAR = \frac{Na^+}{\sqrt{Ca^{2+} + Mg^{2+} / 2}}$$

Organic carbon (OC) was determined using the Walkley-Black wet digestion method and was expressed in percentage (Allison (1965).

3. Results and discussion

3.1. Initial and final soil properties

Table 2a. Initial chemical and physical properties of soil

Ece	pH	Exchangeable cations				OC (%)	SAR	ESP (%)	Na ⁺ /K ⁺ ratio
		Ca ²⁺ (Cmol/kg)	Mg ²⁺ (Cmol/kg)	K ⁺ (Cmol/kg)	Na ⁺ (Cmol/kg)				
0.4	7.5	11.5	9.5	10.2	9.8	5.1	3.02	4.3	0.9

Table 2b. Initial chemical and physical properties of soil

Particle size			
Silt	Sand	Clay	Textural class
10.9	54.2	34.8	Sand clay-loam

Table 3a. Final chemical and physical properties of soil

Exchangeable cations									
Ece	pH	Ca ²⁺ (Cmol/kg)	Mg ²⁺ (Cmol/kg)	K ⁺ (Cmol/kg)	Na ⁺ (Cmol/kg)	OC (%)	SAR (Cmol/kg)	ESP (%)	Na ⁺ /K ⁺ ratio
5.7	7.1	21.5	11.2	12.48	31.1	3.3	7.7	9.6	2.5
3.6	7.1	19.3	10.7	12.63	24.8	3.2	6.4	8.1	1.9
0.7	7.1	21.9	10.8	12.69	10.9	3.4	2.7	4.0	0.9

Table 3b. Final chemical and physical properties of soil

Particle size			
Silt	Sand	Clay	Textural Class
16.9	66.9	16.1	Sandy clay loam
12.7	67.9	19.3	Sandy clay loam
14.7	69.2	16.1	Sandy clay loam

3.2. Soil characteristics as determined during the experiment

The chemical and physical properties of the soil were analyzed before and after the experiment and the results are presented in Tables 1 and 2. The initial electrical conductivity (ECe) of the soil was 0.4 dsm⁻¹ while the pH was 7.5 while all exchangeable cations recorded low values. Similarly, the sodium adsorption ratio (SAR) and exchangeable sodium percentage (ESP) of the soil were also low (0.9 and 0.1 % respectively) in the initial soil sample. Increased salinity levels influenced all the physical and chemical properties of the initial samples as well as the SAR and ESP values (Table 3). The final ECe, SAR, ESP and exchangeable cations were all increased at the end of the experiment, but soil pH decreased with increase in NaCl concentration. The soil Na⁺ increased with increase in NaCl concentration. This was the result of accumulated effect over time (KhajehHosseini et al., 2003; Farhoudi et al., 2007). Potassium, Magnesium and Calcium also increased. This was an indication of the impact of the application of farm yard manure on soil nutrient replacement. According to Tasneem et al. (2004) and Tolessa and Friesen (2001), FYM can provide adequate and balanced supply of nutrients just like inorganic fertilizers. There were improvements in soil physical and chemical properties, which could be due to the incorporation of farm yard manure. Ould Ahmed et al. 2010 reported that generally, soil physical, chemical, and biological properties were improved when they incorporated manures into soils. Also, other researchers have reported the beneficial effects of animal manure on soil

structural quality, by reducing bulk density, increasing porosity, water infiltration rate, saturated hydraulic conductivity and others (Hati et al. 2007; Fares et al. 2008).

There is a serious problem associated with the use of saline irrigating water for crop production; this does not only concern the crop response to irrigation water but rather the long-term changes on the soil properties that might seriously modify the soil fertility. Alobaidy et al. (2010) reported that the use of irrigation water with a high Na^+ concentration causes high accumulation exchangeable Na^+ around soil particles". Excess sodium on adsorption site is hazardous to plant health which affects the growth and yield of crops. Darwish et al. (2009) also stated that "almost every aspect of the plant's physiology and biochemistry is affected by soil salinity".

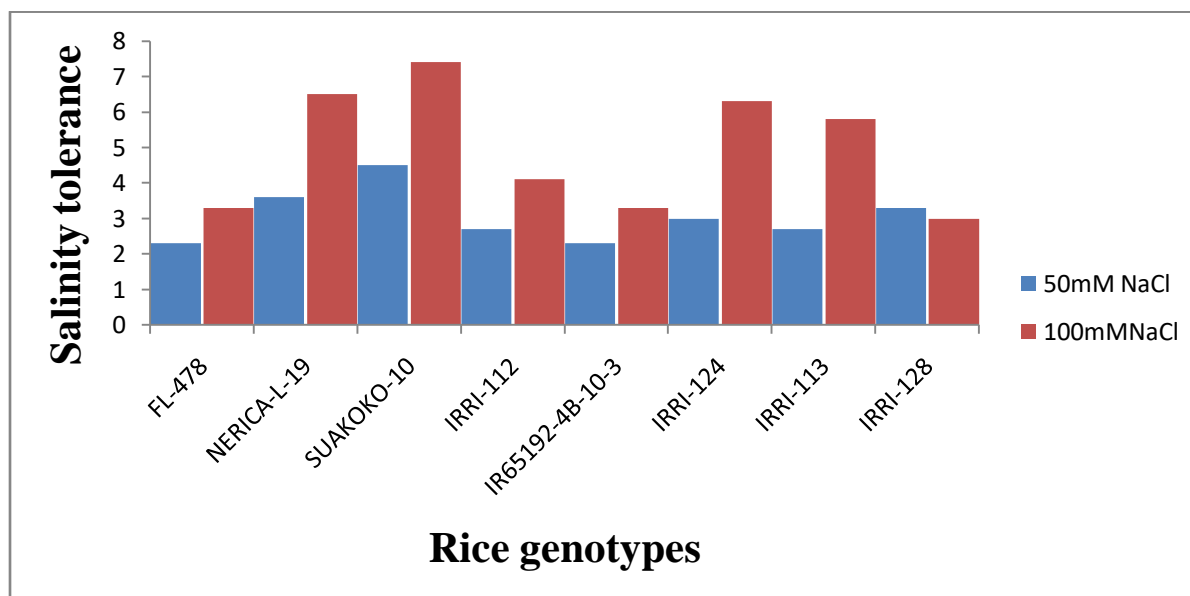


Figure 1. Modified standard evaluation score (SES) of visual salt injury at seedling stage

Note: 1= normal growth (highly tolerant) and 9 = all plants completely dead (highly susceptible)

3.3. Ranking of genotypes on the basis of salt injury at the seedling stage

The salinity tolerance scores calculated for eight rice genotypes are shown in Figure 1. All the 8 rice genotypes grew healthily in the non-salinized condition. In salinized condition, the genotypes showed nearly normal growth at lower NaCl concentration (50mMNaCl) from score 3 to 4.5, but at higher NaCl concentration (100mMNaCl) there showed a wide range of phenotypic variations from score 3 (Nearly normal growth) to 7 (Complete cessation of growth) as shown in Figure 1. The most salinity tolerant genotypes based on the SES scores were FL478, IRR128, IR65192-4B-10-3 and IRR1 112; while the salinity susceptible genotypes based on SES scores were Suakoko-10, NERICA-L19, IRR1 124 and IRR1 113. Islam et al., (2007) made a similar observation on a wide variation in phenotypes from tolerant (score 3) to highly susceptible (score 9) rice lines using modified SES of IRR1 standard protocol. The susceptible genotypes were more stressed under saline condition than tolerant genotypes, as a result ion effects.

Table 4. The Pearson’s correlation coefficients for physiological traits of rice genotypes

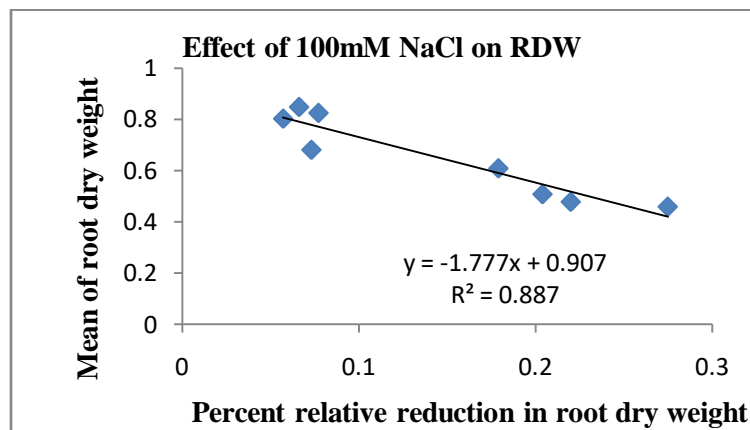
	Plant height (cm)	RDW (g)	SDW (g)	root /shoot ratio
Plant height				
RDW	0.89**			
SDW	0.56**	0.81**		
Root/Shoot Ratio	0.98**	0.88**	0.54**	
SES scores	-0.81**	-0.82**	-0.64**	-0.81**

Note: RDW– roots dry weights; SDW– shoots dry weights

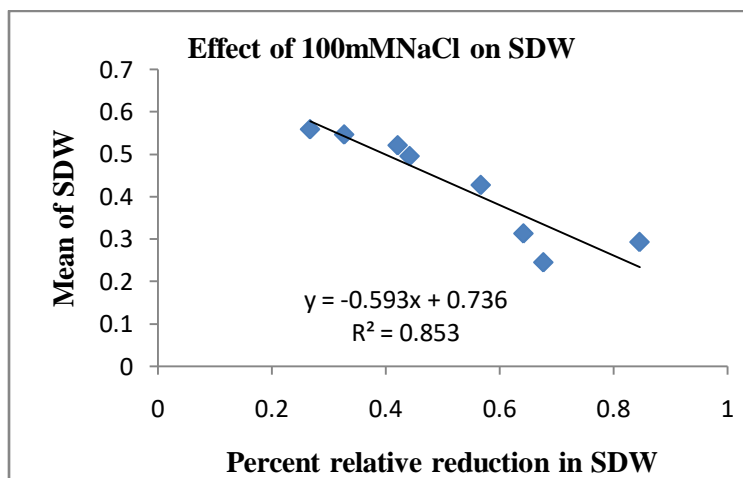
** Correlation was significant at the $p < 0.01$.

3.4. Relationship among various physiological traits of rice genotypes

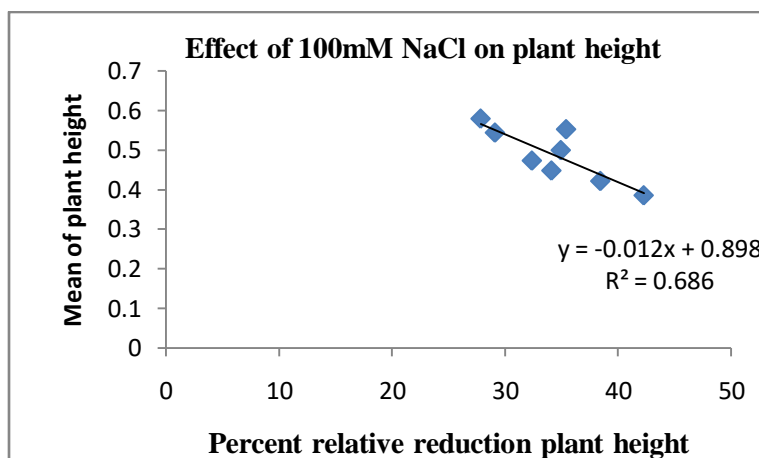
The salinity tolerance scores had significant negative correlation with the entire morphological traits investigated which includes plant height, SDW, SDW and root shoot ratio) as shown in Table 4. Each physiological trait (SDW, RDW, and plant height and root/shoot ratio) correlated positively with each other. All physiological traits showed highly significant positive correlation with each trait except root/shoot ratio & SDW and SDW & plant height which showed a moderately positive correlation with each other. The inverse correlation between scores and the other physiological traits is the result of the inhibiting effects of salinity on root and shoot elongation which leads to the reduction in water uptake by the plant and subsequently reduces plant height and dry matter accumulation. Marcum et al., (2005) reported that the adverse effects of salinity stress on two grasses studied were more obvious on shoot than the root growth. Jamil and Rha (2007) observe a decrease in shoot length, root lengths and dry weights with increasing salt stress.



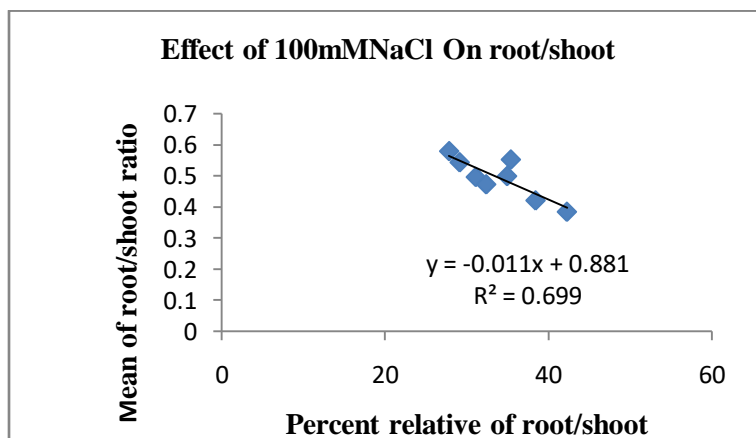
-a-



-b-



-c-



-d-

Note: Plant height (cm), dry weight (g)

Figure 2 (a-d). Effects of salinity on growth and plant characteristics

Table 5. Salinity Tolerance Index at 100mMNaCl concentration

Genotypes	plant height	SDW	RDW	R/S Ratio	Mean of tolerance index value	Tolerance
FL-478	61.55	0.65	0.46	0.61	15.82	T
IRRI-128	57.93	0.66	0.33	0.57	14.87	T
IR65192-4B-10-3	55.29	0.75	0.37	0.5	14.23	T
IRRI-112	52.78	0.68	0.36	0.52	13.59	MT
IRRI-113	50.13	0.5	0.25	0.5	12.84	MS
IRRI-124	45.7	0.45	0.21	0.45	11.70	S
NERICA-l-19	44.85	0.59	0.26	0.44	11.53	S
SUAKOKO-10	42.16	0.44	0.26	0.42	10.82	HS

Note: RDW=Root dry weight; SDW = Shoot dry weight; R/S = Root-Shoot ratio; higher means indicate tolerance and lower means indicate susceptibility. Mean of tolerance index value for genotype was calculated as the average of all indices calculated for the morphological traits of each rice genotype.

Table 6. Salinity susceptibility index for physiological parameters (SSI)

Genotypes	Plant height	RDW	SDW	Root/shoot ratio	Mean	Tolerance
FL-478	0.79	0.79	0.80	0.78	0.79	T
IR65192-4B-10-3	0.92	0.92	0.57	1.00	0.85	T
IRRI-112	0.97	0.93	0.73	0.96	0.90	T
IRRI-113	1.02	1.11	1.15	1.01	1.07	S
IRRI-124	1.11	1.17	1.27	1.10	1.16	S
IRRI-128	0.86	0.98	0.79	0.85	0.87	T
NERICA-L-19	1.13	1.09	0.95	1.12	1.07	S
SUAKOKO-10	1.18	1.08	1.30	1.17	1.18	S

Note: The higher the mean the susceptible the genotype at 100 mMNaCl; genotypes which scored below 1.0 were considered tolerant and those which scored above 1.0 were considered susceptible.

3.5. Ranking of rice genotypes based salinity indices

Rice genotypes were ranked on the basis of their tolerance, susceptibility and the percent reduction in physiological traits observed under salt stress. The Relationships of the percent relative reduction in root dry weights (RDW), shoot dry weight (SDW), root-shoot ratio and plant height under saline condition (100 mMNaCl) to the salinity susceptibility index (SSI) are shown in Figures 2 (a-d). A strong relationship was observed between the mean root dry weight and SSI as shown in (a); the shoot dry weight and SSI in (b) also showed a strong relationship. There were also strong relationships between root-shoot ratio and SSI as well as mean plant height and SSI (Figures c and d). The co-efficient of determination shows that 88.7 % of variation in relative root dry weight can be attributed to salinity susceptibility index and 85.3 % of variation relative shoot dry weight can also be attributed to salinity susceptibility index. In the case of relative root-

shoot ratio and relative plant height, shows that 69.9% and 68.6% of the variation in root-shoot ratio and plant height can be explained by SSI respectively. In this study, the differences among the genotypes with increase in salinity level were much obvious as indicated by the reduction in physiological traits and the results of the various salinity indices (Tables 5 and 6, and Figure 2). Reduction in dry matter accumulation is directly proportional to increased salinity levels (Tsuda and Hirai, 2007).

This was obvious in the susceptible rice genotypes which received higher reductions for all traits studied with increase in salinity levels as compared to the tolerant rice genotypes (Figure 2). The result of this study agrees with that reported by Majkowska et al. (2008). This result is in line with the report of Masood et al., (2005) who suggested that "salt stress reduced the biomass of rice".

On the basis of tolerance and susceptibility indices (Tables 5 and 6), four genotypes were selected as tolerant (FL-478, IRRI-128, IR65192-4B-10-3 and IRRI-112), while the remaining four were considered susceptible to salinity stress (NERICA-L-19, Suakok-10, IRRI113 and IRRI 124). The roots of plants were in directly in contact with the growth media containing toxic salts that retarded the root development, shoot elongation and dry matter accumulation. The result of this study agrees with Syvertsen et al. (2000) and Kasukabe et al. (2006) who reported that under salinity condition, CO₂ assimilation of plant which is a major energy source for growth and development, becomes decreased. Also as reported by Vasquez et al. (2006), reduction in root length caused the decrease in biomass which was observed under salt stress. A decrease in root length and root dry weight with increase in salinity in the present study confirms the results of Syvertsen et al. (2000) and Kasukabe et al. (2006).

4. Conclusion

There was variability in the performance of rice genotypes. Considering the phenotypic observation and the three indices used, salinity stress affected all Morpho-physiological and biochemical parameter of rice genotypes. The results indicate that three of the rice genotypes from IRRI (FL 478, IRRI 128 and IR6592-4B-10-3) were salinity tolerant and one (IRRI 112) was moderately tolerant to salinity stress. The two rice genotypes from AfricaRice and two of the genotypes from IRRI, (NERICA-L-19, SUAKOKO-10, IRRI 113 and IRRI124) were susceptible to salinity stress respectively. Therefore, the two rice genotypes from AfricaRice (NERICA-L-19 and SUAKOKO-10) were selected as recurrent parents and two of the rice genotypes from IRRI (FL-478 and IR65192-4b-10-3) were selected as donor parents to be used in a breeding program.

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