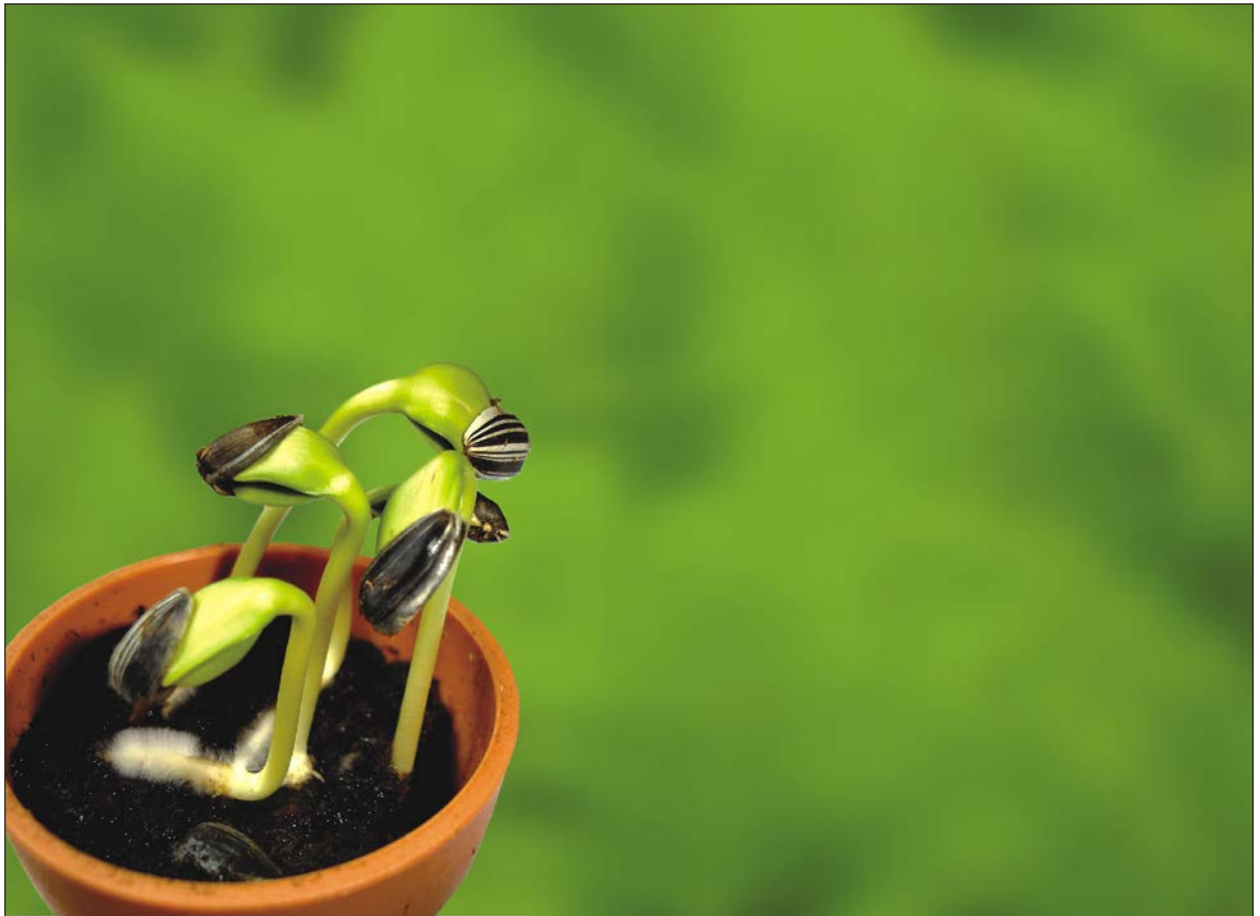




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Research Article

Phenotypic Variability of 20 Rice Genotypes Under Salt Stress

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Abstract

Salinity is a serious problem holding-back rice production in Niger. To identify a tolerant genotype it is important to exploit the genetic variability of the available germplasm. A greenhouse pot experiment was conducted to determine the response of rice varieties under salt stress. Fifteen exotic and five local varieties were evaluated in a under four salt levels and a control without salt. The experimental design was a split plot with two replicates. Significant genotype by salt concentration effect was observed. Significant variability among varieties ($p = 0.001$) across and within salt levels was present for all the traits measured. Five salt tolerant genotypes with high selection index were identified.

Key words: Salinity, tolerance, genetic diversity, till number, green leave number, penical weight

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INTRODUCTION

Salinization is the accumulation of water-soluble salts in the soil to a level that impacts on agricultural production, environmental health and economic welfare (Rengasamy, 2006). Salinity is a worldwide problem of serious nature in arid and semi-arid regions where, most of the developing countries happen to fall (Ismail *et al.*, 2007). It is one of the most important factors in reducing crop yields in most countries in the world (Rengasamy, 2006; Gholizadeh and Navabpour, 2011). FAO (2003) reported that nearly 50% of the irrigated lands in arid and semi-arid regions of the world have some degree of soil salinization problems. This is more serious since irrigated areas are responsible for one-third of world's food production (Linh *et al.*, 2012).

Plant growth on these soils is adversely affected because of reduced water uptake, salt toxicity and nutrient imbalances (Ismail *et al.*, 2007). Salt stress limits rice (*Oryza sativa*) production in vast areas worldwide and the problem is ever increasing because of irrational human acts, causing secondary salinization as well as because of global warming (Moradi and Ismail, 2007). For rice, soil salinity beyond $EC_e \sim 4 \text{ dS m}^{-1}$ is considered moderate salinity while, more than 8 dS m^{-1} becomes high (Singh, 2009). Soil solutions high in sodium chloride with electrical conductivity are associated with 50% decrease in yield (Moormann and van Breemen, 1978) because of both osmotic and ionic stresses (Siringam *et al.*, 2009).

Plant scientists have adopted various strategies to overcome the salinity. One of them is to exploit the genetic variability of the available germplasm to identify a tolerant genotype (Ashraf *et al.*, 2006). Existence of appropriate genetic variation is a prerequisite for the improvement of any character, through selection and breeding (Mahmood *et al.*, 2009). Fortunately, diversity in salt tolerance has been found in a considerable number of rice varieties (Bari and Hamid, 1988; Mishra *et al.*, 2000; Zeng *et al.*, 2002). The screening of rice genotypes is necessary to identify the salt tolerant germplasm for breeding programs (Akram *et al.*, 2010). Study on the response of rice to salinity stress may be helpful in breeding salt tolerant cultivars. The objectives of this study were to:

- Determine the variability of rice genotypes in response to salinity tolerance
- Assess the performance of rice genotypes under salt stress
- Identify and select promising salt tolerant genotypes as parents for a development of new varieties with salinity tolerance

MATERIALS AND METHODS

The experiment was conducted at the Regional Agronomic Research Centre (CERRA) of Kollo in greenhouse. Kollo is geographically located at latitude $13^{\circ}19'43''\text{N}$, longitude $2^{\circ}19'16''\text{E}$ and 250 m altitude.

The plant material was composed of 15 genotypes known as salt tolerant from IRRI and five farmer's varieties from INRAN (National Institute for Agronomic Research of Niger). These were: IRGC 30, IRGC 249, IRGC 15963, IRGC 17038, IRGC 17041, IRGC 17229, IRGC 26612, IRGC 37084, IRGC 37179, POKKALI, NSIC RC 106, IRRI 126, IRRI 113, IRRI 124, CSR 36, GAMBIAKA, IR 1529-680, NERICA-L-49, GIZA 175 and BG 90-2.

The soil transported from the rice salt free field was clayey and was composed of 52.8% clay, 30.4% silt and 16.7% sand. The Sodium Adsorption Ratio (SAR) and Potassium Adsorption Ratio (PAR) were equal and very low (0.06). The exchangeable sodium percentage was about 6% and the electrical conductivity of 0.12 dS cm^{-1} . The water used for irrigation had a sodium percentage of 1% and electrical conductivity of 1.42. The total dissolved salts were 14.15 meq L^{-1} . The SAR and PAR were 0.013 and 0.008, respectively.

Two hundred round pots of 20 cm diameter were used for the experiment. Each pot was filled with 4 kg of dried soil. The watering was done using tap water. Seeds of 20 entries were pre-germinated before transplanting in pots. Twenty Days After Seeding (DAS), seedlings were thinned to two per pot and the water level was raised to about 1 cm above soil. The water level was maintained daily and the plants were protected from any pests and diseases by spraying insecticide and fungicide. The first fertilization was done at 20 DAS using NPK 15-15-15 at a rate of 2 grams per pot. The second fertilization was done at 58 DAS with urea at the rate of 2 g pot^{-1} .

Salinization was done by adding to the soil a salinized solution, which was obtained by adding table salt to water up to desired Electrical Conductivity (EC) which equals to 10 and 12 dS m^{-1} . An Atago Digital EC Meter (DEC-2) was used to calibrate the desired level of electrical conductivity. Salinization of water was made using the following methods:

- The 5 and 6 g NaCl L^{-1} gives an EC of 10 and 12 dS m^{-1} , respectively (Gregorio *et al.*, 1997)
- The 6.4 and 7.68 g NaCl L^{-1} gives an EC of 10 and 12 dS m^{-1} , respectively (Shannon *et al.*, 1998)

When the seedlings were 28 days old, all the water in the pots was drained out from pots. Then the pots were filled up with salinized water solution. The water level was maintained

daily (5 cm above soil surface) by adding ordinary water. Untreated soil served as the check.

The experiment design was a standard split plot with two replicates. The treatments were five salt levels: 10 dS m⁻¹ (Shannon), 10 dS m⁻¹ (Gregorio), 12 dS m⁻¹ (Shannon), 12 dS m⁻¹ (Gregorio) and control and 20 rice genotypes. The salt levels were assigned as the main plot factor and the genotypes were assigned as sub-plot factors.

Data recorded at both vegetative and maturity stage were the number of green leaves of each genotype on the main culm, the number of tillers per genotype, panicle number per plant, the panicle weight, flag leaf weight and dry weight. The symptoms of salt stress for each plant were recorded 42 days after salt treatment according to the standard evaluation system used at IRRI (Gregorio and Senadhira, 1995). Weights (Wi) for the selection index were allocated based on the relative importance of each measured trait as an indicator of salinity tolerance (Efisue, 2006). The Selection Index (SI) of each genotype was calculated as:

$$SI = \frac{P_i W_i + P_j W_j + \dots + P_n W_n}{\sum P_i W_i + \sum P_j W_j + \dots + \sum P_n W_n}$$

where, P_i is standardized phenotypic value of the trait observed, W_i is the assigned weight value to the trait in the selection index.

SAS 9.2 software was used for statistical analysis.

RESULTS

The average mean score ranged from 4 (tolerant) to 9 highly susceptible (Table 1). The number of tiller significantly differed among genotypes. Genotypes were significantly and differently affected by salt treatments as far as panicle number is concerned. The panicle number was significantly affected by genotype treatment interaction. In term of panicle weight some evidences showed that there was significant variability among genotypes as well as among treatments. An interaction also existed between salt level and genotypes for this trait. The analysis of variance (Table 2) also showed that biomass produced greatly varied among genotypes and also among treatments.

The Tiller Number (TN) and Green Leave number (GL) had strong positive association (Table 3). Genotypes that had good tillering ability had also the ability to keep longer their leaves green. But both had weak positive association with all the other traits. A strong significant positive association also existed among the PN, PW and FIW. In terms of tillering ability under salt stress, genotypes means comparison (Fig. 1) displayed four groups the most performing being IRGC 17229, IRRI 126 and IRRI 113. The second group was composed of IRRI 126, IRRI 113, IRGC 30 and Pokkali.

The means comparison across genotypes (Table 4) also showed that Pokkali, IRRI 126, IRGC 17229, IRRI 113 and NSIC RC 106 had significant ability to keep more green leaves in salt condition. The IR1529 and GAMBIKA had poor performance of bearing functional leaves in salt condition. Significant diversity existed among genotypes concerning this trait. Number of panicle differed significantly among genotypes within and across treatments. Means comparison showed that panicle number differed significantly among genotypes across treatments. There was clear evidence that the best genotypes concerning panicle number were IRRI 113, IRRI 126, IRRI 124 and NSIC RC 106.

Table 1: Genotypes behavior under salt stress based on visual scoring

Genotypes	Scoring	Tolerance
Pokkali	4	Tolerant
IRRI 126	4.5	Tolerant
IRRI 113	4.8	Tolerant
IRGC 17229	5.5	Moderately tolerant
NSIC RC 106	5.5	Moderately tolerant
GIZA 175	6	Moderately tolerant
IRGC 17041	6.1	Moderately tolerant
IRRI 124	6.8	Moderately tolerant
CSR 36	6.8	Moderately tolerant
GAMBIKA	7	Susceptible
NERICA L49	7	Susceptible
BG 90-2	7.3	Susceptible
IRGC 17038	7.5	Susceptible
IRGC 30	7.8	Susceptible
IRGC 249	8	Susceptible
IRGC 37179	8	Highly susceptible
IRGC 37084	8.5	Highly susceptible
IRGC 15963	8.7	Highly susceptible
IRGC 26612	9	Highly susceptible
IR 1529-680	9	Highly susceptible

Table 2: Analysis of variance of traits

Source	DF	TN	GLN	PN	PW	DW
Replication	1	364.59	15.68	450	415.64	4433.05
Treatment	4	7093.36***	680.48***	1728.35**	3000.44**	15276.94**
Replication × treatment	4	54.05 ^{ns}	51.22**	170.85 ^{ns}	191 ^{ns}	1452.59 ^{ns}
Genotype	19	1039.02 ***	261.88**	4682.70**	6350.37**	36080.92**
Treatment × genotype	76	1453.31 ^{ns}	236.32 ^{ns}	5685.45*	7667.09*	46630.85 ^{ns}
Error	94	1691.31	220.10	3894.15	5391.37	32169.40

*, ** and ***Significant at 0.05, 0.01 and 0.001 probability level respectively, TN: Tillers number, GLN: Green leaves number, PN: Panicle number, DW: Dry weight, DF: Degree of freedom, ns: non significant

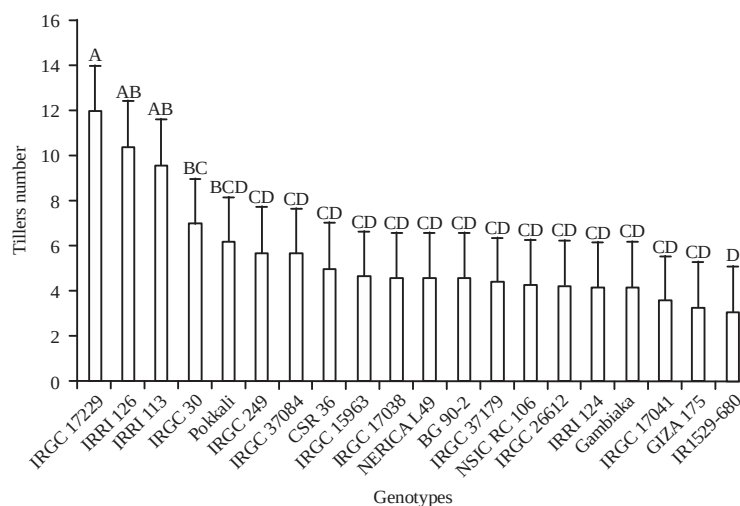


Fig. 1: Genotypes tillers number means comparison

Table 3: Correlation coefficients among studied traits of 20 rice genotypes

Traits	GL	TN	PN	PW	FIW
TN	0.70 **				
PN	0.29 *	0.29*			
PW	0.32 *	0.30*	0.90***		
FIW	0.35*	0.28*	0.70**	0.70**	
DW	0.29 *	0.23*	0.88 ***	0.86**	0.75**

*, ** and ***Significant at 0.05, 0.01 and 0.001 probability level respectively, TN: Tillers number, PN: Panicle number, GL: Green leaves number, PW: Panicle weight, FIW: Flag leaf weight, DW: Dry weight

Table 4: Means comparison of green leaves number and panicle number

Genotypes	Green leaves number	Group	Genotypes	Panicles number	Groups
Pokkali	5.1	A	IRRI 113	14.7	A
IRRI 126	4.6	AB	IRRI126	12	AB
IRGC 17229	4.3	AB	IRRI 124	11.4	BC
IRRI 113	4.1	ABC	NSIC RC 106	7.7	BCD
NSIC RC 106	4.1	ABC	IRGC 30	7	BCD
GIZA 175	4.0	ABC	GIZA 175	6.7	BCD
IRGC 17041	3.5	ABCD	Pokkali	6.3	BCD
IRGC 17038	3.4	ABCD	CSR 36	6.2	CD
CSR 36	3.3	ABCDE	NERICA L49	6	CDE
NERICA L49	3.2	BCDE	IRGC 17041	4.8	DE
IRRI 124	3.1	BCDE	IR1529-680	4.3	DE
IRGC 30	2.6	CDEF	GAMBIAKA	3.6	DE
BG 90-2	2.6	CDEF	IRGC 17038	3.5	DE
IRGC 37084	2.5	CDEF	IRGC 37084	3.2	DE
IRGC 249	2.4	CDEF	IRGC 15963	1.1	E
IRGC 37179	2.4	CDEF	IRGC 26612	1.1	E
IRGC 15963	1.9	DEF	BG 90-2	1	E
IRGC 26612	1.5	EF	IRGC 17229	1	E
Gambiaka	1.5	EF	IRGC 249	0.5	E
IR 1529-680	1.0	F	IRGC 37179	0.5	E
LSD _{0.01} = 1.8			LSD _{0.01} = 5.684		

NB: Genotypes followed by the same letter are not significantly different

Genotypes means comparison (Table 5) showed that there was strong evidence that IRRI 113, IRRI 126, IRRI 124 and NSIC RC 106 were genotypes that bore the heaviest panicles. However, they shared the same group with CSR 36, Pokkali

and Giza 175. The top group of genotypes in term of biomass included IRRI 126, Pokkali, IRRI 113 and CSR 36. Genotypes that produced lesser dry weight were IRGC 249, IRGC 37179, IRGC 17229, IRGC 26612 and IRGC 15963.

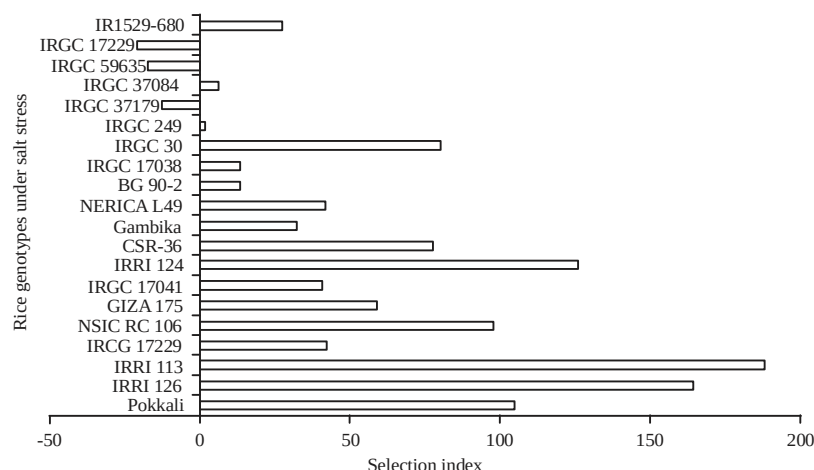


Fig. 2: Selection index of 20 rice genotypes under salt stress

Table 5: Genotypes panicle weight and dry weight means comparison

Genotypes	Panicle weight (g)	Groups	Genotypes	Dry weight (g)	Groups
IRRI 113	19.50	A	IRRI 126	46.313	A
IRRI 124	17.39	AB	Pokkali	38.1625	AB
IRRI 126	12.97	ABC	IRRI 113	37.716	ABC
NSIC RC 106	12.66	BC	CSR 36	30.327	ABCD
IRGC 30	8.79	CD	IRGC 30	29.391	BCDE
CSR 36	8.64	CD	IRRI 124	25.553	BCDEF
Pokkali	8.06	CD	GIZA 175	22.561	BCDEFG
GIZA 175	6.68	CD	NSIC RC 106	22.435	BCDEFG
IR1529-680	5.36	D	IRGC 17041	21.551	CDEFG
IRGC 17229	4.91	D	NERICA L49	17.465	DEFGH
GAMBIAKA	4.87	D	GAMBIAKA	17.372	DEFGH
BG 90-2	4.25	D	IR1529-680	16.492	DEFGHI
IRGC 249	4.25	D	IRGC 17038	13.118	EFGHI
NERICA L49	3.71	D	BG 90-2	9.269	FGHI
IRGC 17041	3.12	D	IRGC 37084	7.566	GHI
IRGC 37179	1.75	D	IRGC 15963	3.637	HI
IRGC 37084	1.47	D	IRGC 26612	3.446	HI
IRGC 17038	0.77	D	IRGC 17229	1.188	HI
IRGC 26612	0.18	D	IRGC 249	0.25	I
IRGC 15963	0.15	D	IRGC 37179	0.225	I

LSD 0.05 = 6.7

LSD_{0.05} = 16.43

Genotypes with the same letter are not significantly different

The selection index value ranged from -20.68-187.5 (Fig. 2). Based on selection index the best five genotypes were IRRI 113, IRRI126, IRRI 124, Pokkali and NSIC RC 106 with respectively a value of 187.5, 164, 125.5, 104.4 and 97.5.

Principal component analysis showed that the first two Principal Components (PC1 and PC2) explained 67 and 17% of the total variation, respectively. The main traits that had the highest loading scores (and hence contributed most for the differences) were the scoring index, panicle number, panicle weight and dry weight for component 1, tiller number and green leaves for component 2. These components were used to draw a dendrogram with single linkage and euclidean distance (Fig. 3). At a distance of 3.96 thirteen clusters were

distinguished. The local genotypes (Gambiaka, IR15 and NERICAL49) were in the same cluster. At a distance of 7.92 five groups were separated. Pokkali, IRRI 126, IRRI 113 were each and alone in separate group.

DISCUSSION

All the tolerant genotypes observed came from IRRI. These included Pokkali known to be highly salt tolerant (Xie *et al.*, 2000; Gregorio *et al.*, 2002; Ismail *et al.*, 2007). However, some IRRI genotypes, known as salt tolerant showed susceptibility. This may be due to confined experimental conditions that may not be conducive to root growth. The

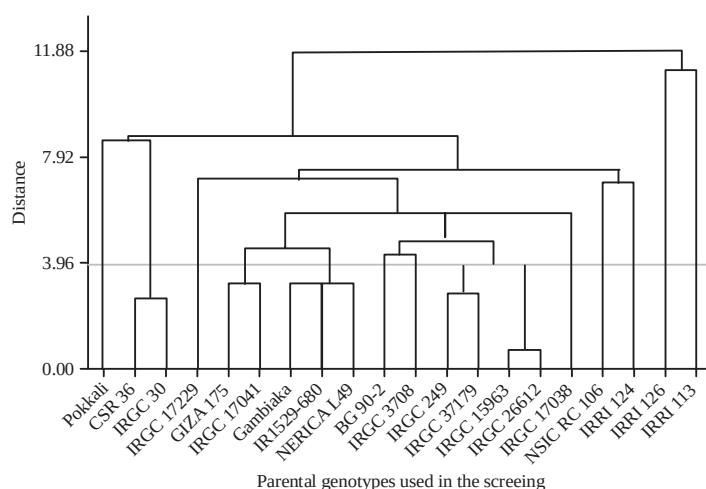


Fig. 3: Dendrogram of 20 parental genotypes

score based on visual symptoms related well to grain yield and yield reduction due to salt stress (Ray and Islam, 2008).

Results indicated salinity had a profound influence on plant tillers number. Effects of salinity on the number of tillers were reported by researchers (Asch *et al.*, 2000; Neves-Piestun and Bernstein, 2001) that found a reduction of tiller number under salt stress. High salinity affects mostly all aspects of plant physiology and metabolism (Zhu, 2002) including germination, vegetative growth and reproductive development (Munns, 2002). Reductions in tiller number per plant and spikelet number per panicle were the major causes of yield loss (Zeng and Shannon, 2000). There was genotypic variation in the response of these genotypes to salinity in terms of tiller number.

Genotypes under salt conditions bore fewer green leaves than controls. This was due to salt affect because salinity induces leaf senescence (Yeo and Flowers, 1983; Leidi *et al.*, 1991; Yeo *et al.*, 1991) and reduces photosynthetic leaf area of a plant to a level that cannot sustain growth (Munns, 2002; Rad *et al.*, 2012). In rice, concentration of salt in leaves is found to cause different degrees of toxicity in different varieties, which is termed tissue tolerance (Yeo and Flowers, 1983; Ismail *et al.*, 2007).

Panicle weight was decreased by salinity compared to the control, because, according to Asch and Wopereis (2001), salinity in reproductive stage, decreases the number of filled panicles, fertile panicle, weight of 100 grains, percentage of fertile grains and increases fertile tillers. Tiller number per plant and spikelet number per panicle contributed the most variation in grain weight per plant under salinity.

All genotypes that had no tillering ability, little or no green leaves and lacked panicles were also scored high these

were the susceptible genotypes. Some have more tillers, more green leaves and less panicle weight. This is the result of tolerance at vegetative stage and susceptibility at reproductive. This may be due either to tiller non productivity (tillers did not flower) or florets abortion. The tolerant genotypes at vegetative stage had highest dry matter quantity. Tolerant genotypes had high positive selection index and susceptible genotypes had low or negative selection index.

The dendrogram showed that at a distance of 11.88 the two best genotypes (IRRI 113 and IRRI 126) were in the same group when all the others appeared in one other group. These two genotypes seem to be related. The local varieties (GAMBIAKA, IR1529 and NERICA49) appeared also in the same cluster. This implied their relatedness.

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