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Screening *Oryza Sativa* L. for Salinity Tolerance During Vegetative Stage for the Coastal Region of Niger-Delta Nigeria

Andrew Abiodun Efisue*, Ella Elizabeth Igoma

Department of Crop & Soil Science, University of Port Harcourt, Port Harcourt, Rivers State, Nigeria

Email address:
andyefisue@yahoo.com (A. A. Efisue)
*Corresponding author

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Abstract: Background and Objective: The coastal region of Niger Delta of Nigeria is favourable for rice production because of the swampy nature, but rice productivity is very low due high level of salinity in the soil. The objectives of this study was to identify promising salt tolerant genotypes for the deployment into this salt stressed region and also for population improvement for salt breeding programme. Materials and Methods: Two potted experiments comprise the same twenty genotypes were established concurrently in coastal soil and controlled sterilized top soil in two replications in a randomized complete block design at the University of Port Harcourt teaching and research farm. Results: Most of the genotypes showed differential response to salinity stress, the protocol used for the screening could be effective as significant difference was observed based on t-test for the two experiments. Salinity scores showed significan negetative association with number of tillers and leaf area index (LAI). Principal component 1 contributed 44.21% and 38.44% of the total variation in the control and coastal soil experiments, respectively. Conclusion: Genotypes IR 84105-5-B-1-B-3 and IR 84105-5-B-1-B-2 maybe promising for deployment into this salinity stressed region. The leave area index and tillering ability of the rice crop are major yield components, their high weight and factor loading values indicate that they are purtative traits for salinity tolerant, which could be used for population development in salinity breeding programme.

Keywords: Oryza Sativa L., Salinity, Genotypes, Coastal Soil, Principal Component

1. Introduction

Rice is a staple crop in Nigeria and consumption rate increases appreciably across the social class of Nigerians, however, the domestic production cannot meet up the demand. Nigeria is endowed with favourable rice ecologies especially the swamp rice ecology, which contributed about 52 % of the total rice production of the country [1]

The Niger Delta region stretch along the coast of Nigeria favourable for swamp rice cultivation. Rice production in this region is very poor due to the salinity of the soil received from the upsurge of the coastal water. Potentially, this region has the capacity of producing rice that could match Nigerian consumption.

Rice is regarded as the most suitable cereal crops adapted to salinity stressed environments, however, it shows moderate reactions to salinity stress [2, 3] especially at the vegetative stages of the crop [2, 4]. Salinity as an abiotic stress under the influence of complex traits like drought [5] and conferment of stress is genotype dependence, duration , severity of stress and stages of crop phenology [6, 7] . Sensitivity of rice at the vegetative leads to yield reduction of more than 50% in severe situation [8-10].

Scoring rice genotypes in response to salinity stress is very complex, however, visual symptoms is still regarded as the most appropriate method for rice salinity screening [11].

Yield components are related to the final grain yield [11, 12]. Gregorio et al. [11] reported reduction in plant height, root length, tillering ability and biomass under salinity stressed conditions.

Therefore, screening for rice tolerant varieties requies good understanding of the crop physiology as salinity interfer with the uptake of essential nutrient [13]. Zhong et al. [14] reported rice quantitative trait locus SKC1 for sultant tolerance encodes that sodium transporter, the physiological analysis also suggest that SKC1 maybe involved in regulating K+ / Na+ balance under stress as imbalance adversely affected grain yield [15], thus providing a potential tool for salt tolerance rice improvement.

The development of salt-tolerance rice varieties will be a means of expanding rice production in salt affected environments [16] as in built salt tolerance varieties are the mos promising and economically viable [17] . The objectives of this study is therefore to identify salt tolerant rice for the deployment into stressed environments and as donors for population improvement for salt breeding programme.

2. Materials and Methods

Two experiments were established at the University of Port Harcourt, Faculty of Agriculture Teaching and Research Farm. The experimental site has an average temperature of 28 to 30^{0} C and annual rainfall ranging from 2000 to 2680 mm. This experiment comprises twenty genotypes including nineteen advance breeding lines and Pokkali as salt-tolerant check, were evaluated in this study as indicated in Table 1.

Table 1. *Genetic materials used in the experiments.*

S/N	Lines	Sources
1	IR84105-5-B-1-B-1	IRRI
2	IR84105-5-B-1-B-2	IRRI
3	IR84105-5-B-1-B-3	IRRI
4	IR84105-5-B-1-B-4	IRRI
5	IR84105-5-B-1-B-5	IRRI
6	IR84931-9-B-1-B-1	IRRI
7	IR84931-9-B-1-B-2	IRRI
8	IR84931-9-B-1-B-3	IRRI
9	IR84931-9-B-2-B-1	IRRI
10	IR84931-9-B-2-B-2	IRRI
11	IR84931-9-B-2-B-3	IRRI
12	IR84931-9-B-2-B-4	IRRI
13	IR84931-9-B-2-B-5	IRRI
14	IR84961-11-B-1-B-1	IRRI
15	IR84961-11-B-1-B-2	IRRI
16	IR84961-11-B-1-B-3	IRRI
17	IR84961-11-B-1-B-4	IRRI
18	IR84961-11-B-1-B-5	IRRI
19	IR83407-6-B-11-B-1-B-1	IRRI
20	POKKALI	IRRI

2.1. Experimental Design and Layout

Two experiments were established concurrently in randomized complete block design in two replications in pots. Experiment one is the control planted in a sterilized top soil of average Electrical Conductivity (EC) 0.45 dS/m of salinity. Experiment two: soil samples of about 30 cm depth were randomly collected from Niger Delta coastal region and filled into pots. The coastal soil has an average of electrical conductivity (EC) 2.75 dS/m of salinity. The genetic materials used in experiment one was replicated in experiment two. The Electrical conductivity meter by HANNA instruments model HI 9835 was used to calibrate the salinity level of the soils. Planting was done by dibbling four seeds per pot and thinned to two seedlings 15 d after sowing. Irrigation was applied regularly to maintain the soil capacity. Inorganic fertilizer (NPK 15:15:15) was applied in a basal application of 200 kg ha^{-1}(N_2, P_2O_5 and K_2O) and top-dressed with urea (46% N) 65 kg ha^{-1} at tillering and 35 kg ha^{-1} at booting.

2.2. Data Collection

Data was collected at appropriate stage of the crop development. The agronomic characters were measured at weekly intervals. The 'Standard Evaluation System (SES) for Rice' reference manual [18] was used for all trait measurements except where stated otherwise.

Two young fully expanded leaves from the main stem were randomly selected in each pot and leaf area (LA) was determined using a leaf area meter (li-3100, Lincoln, NE USA). Leaf area index (LAI) was calculated as described by Yoshida [19] as follows: LAI = (sum of the leaf area of all leaves /unit area where the leaves have been collected).

Plant height was measured in cm from the plant base to the tip of the highest leaf using meter rule and number of tillers taken at maximum tillering sate of the pant. The first and second salinity scores were taken at 20 d after seeding and 35 days after seeding, respectively.

2.3. Data Analysis

All data were subjected to Analysis of variance (ANOVA) using PROC GLM and PRINCOM of SAS [20], for mean separation and principal component analysis (PCA), respectively.

3. Result

3.1. Agronomic Performance of Genotypes

Plant height had significance variation among the genotype tested at ($P \leq 0.01$). Eight genotypes had an average height above the grand mean of 99.00 cm, average plant height of the genotypes range from 84.17 to 115.17 cm and Pokkali the highest of 125.00 cm (Table 2). Nine genotypes had LAI value below the grand mean values of 1.466 including the check (Pokkali). However, there was a significance difference at ($P \leq 0.05$) among the genotypes for LAI and IR 84105-5-B-1-B-3 had the highest value (2.17). The number of tillers at maximum tillering stage of the crop showed significance difference among the genotypes at ($P \leq 0.01$). Genotypes with high tiller number are IR 84105-5-B-1-B-5 (10), IR 84105-5-B-1-B-1 (10) and IR 84105-5-B-1-B-3 (10). The first salinity score showed genotypes reaction of high grand mean value (3.686) compared to the second salinity score (3.412), while the check showed no difference for the two scores. However, there was significance difference among the genotypes at ($P \leq 0.05$) and ($P \leq 0.01$) for first salinity score and second salinity score, respectively (Table 2).

Table 2. *Agronomic performance of genotypes in the control experiments.*

Lines	Plant height (cm)	LAI	Number of tillers / plant	1st salinity score	2nd salinity score
IR84105-5-B-1-B-1	115.17	1.93	10	4.33	3.00
IR84105-5-B-1-B-2	101.83	1.63	9	3.67	3.00
IR84105-5-B-1-B-3	97.67	2.17	10	3.67	3.33
IR84105-5-B-1-B-4	98.00	1.80	7	3.67	3.33
IR84105-5-B-1-B-5	103.17	1.80	11	3.67	3.67
IR84931-9-B-1-B-1	88.83	1.33	8	3.00	2.00
IR84931-9-B-1-B-2	101.17	1.33	7	4.00	2.33
IR84931-9-B-1-B-3	99.00	1.47	7	3.33	3.33
IR84931-9-B-2-B-1	81.83	0.77	5	3.00	3.67
IR84931-9-B-2-B-2	104.00	1.50	8	3.00	4.33
IR84931-9-B-2-B-3	103.83	1.37	9	3.00	1.00
IR84931-9-B-2-B-4	100.33	1.53	7	3.00	2.67
IR84931-9-B-2-B-5	85.67	1.43	6	4.33	3.67
IR84961-11-B-1-B-1	104.67	1.27	7	3.67	3.33
IR84961-11-B-1-B-2	84.17	0.93	6	3.67	4.33
IR84961-11-B-1-B-3	88.83	1.30	5	4.33	5.67
POKKALI (check)	125.00	1.35	5	5.33	5.33
Mean	99.000	1.466	7.3	3.686	3.412
LSD (0.05)	18.601	0.660	3.27	1.206	2.066
P value	**	*	**	*	**

*, **, and *** significant at 0.05, 0.01 and 0.001 probability level respectively, LAI = leave area index

Genotypes in coastal sol experiment showed significance difference for all the observed traits. Plant height had 13 genotypes above the grand mean (96.110 cm) and IR 84931-9-B-2-B-3 had the highest value (108.83). The number of tillers at maximum tillering stage, 9 genotypes ha more tillers than the Pokkali (8.0). Significance difference at ($P \leq 0.001$) among the genotypes for LAI and IR 84105-5-B-1-B-3 had the highest value (3.40). Salinity scores at first and second reading were higher in value than the control at ($P \leq 0.001$) Table 3.

Table 3. *Agronomic performance of genotypes in the coastal soil experiment.*

Lines	Number of tillers/plant	Plant height (cm)	LAI	1st salinity score	2nd salinity score
IR84105-5-B-1-B-1	15	102.67	2.90	3.00	4.67
IR84105-5-B-1-B-2	12	102.67	3.03	4.67	3.67
IR84105-5-B-1-B-3	15	107.23	3.40	4.33	3.33
IR84105-5-B-1-B-4	11	101.50	2.25	4.00	4.33
IR84105-5-B-1-B-5	9	86.17	1.95	5.33	5.67
IR84931-9-B-1-B-1	8	100.83	2.09	5.33	4.67
IR84931-9-B-1-B-2	7	102.83	1.98	6.33	6.00
IR84931-9-B-1-B-3	8	100.17	1.94	3.00	7.00
IR84931-9-B-2-B-1	7	51.00	1.23	4.50	3.67
IR84931-9-B-2-B-2	7	96.50	1.98	3.00	5.00
IR84931-9-B-2-B-3	7	108.83	2.34	4.00	3.67
IR84931-9-B-2-B-4	6	112.17	2.64	4.00	4.67
IR84931-9-B-2-B-5	12	104.50	1.61	5.67	4.33
IR84961-11-B-1-B-1	12	54.50	1.31	8.00	5.00
IR84961-11-B-1-B-2	9	99.17	1.28	1.33	3.00
IR84961-11-B-1-B-3	6	104.50	0.98	3.67	7.67
POKKALI (check)	8	25.00	0.65	3.00	7.00
Mean	9.5	96.110	1.975	4.307	4.902
LSD (0.05)	5.57	35.825	1.064	1.818	1.894
P value	**	**	***	***	***

, and * significant at 0.01 and 0.001 probability level respectively, LAI = leave area index

All the parameters taken showed negative relationship between the means of the two experiments and significance differences for all observed parameters except plant height and first salinity score. The LAI had the highest t-value (-3.91) (Table 4).

Table 4. *t -Test to compare the relationship between control and coastal experiments.*

Trait	t-value	Probability-value
LAI	-3.91	***
Plant height	-0.88	ns
Number of tillers	-2.32	**
First salinity score	-1.5	ns
Second salinity score	-3.43	**

, and * significant at 0.01 and 0.001 probability level respectively, ns = non-significant

3.2. Principal Component Analysis and the Agronomic Traits

The principal component analysis one (PCA 1) and PCA 2 contributed 44.21 % and 35.885%, respectively of the total variance observed in the control experiment Table 5. The number of tillers and leave area index (LAI) contributed most to the total variance of PCA 1 and plant height and salinity scores for PCA 2. The salinity scores had negative values in PCA 1 Table 5.

Table 5. *Principal component analysis for the control soil experiment.*

Eigenvectors					
Trait	**Prin 1**	**Prin 2**	**Prin 3**	**Prin 4**	**Prin 5**
Ht	0.3466	0.4915	-0.6504	0.4408	-0.1451
LAI	0.563429	0.238616	0.50041	-0.164926	-0.589915
Salt1	-0.11453	0.680265	-0.058712	-0.656492	0.299478
Salt2	-0.3821	0.484154	0.52556	0.574326	0.116072
Till	0.634997	-0.065883	0.21671	0.132952	0.726497

Prin1, Prin1, Prin2, Prin3, Prin4 and Prin5 = Principal Component Analysis 1, 2, 3, 4 and 5, respectively.
Ht = plant height, LAI = leave area index, Till = number of tillers, Salt1 = first salinity score and Salt2 = second salinity.

The PCA 1 and PCA 2 contributed 38.44% and 27.07% of the total variance in the coastal soil experiment, respectively Table 6. The number of tillers and leave area index (LAI) contributed most to the total variance of PCA 1 and plant height and first salinity scores for PCA 2. The salinity scores had negative values in PCA 1, while negative scores were observed for LAI, number of tillers and first salinity scores in PCA 2. Table 6.

Table 6. *Principal component for coastal soil experiment.*

Eigenvectors					
Trait	**Prin 1**	**Prin 2**	**Prin 3**	**Prin 4**	**Prin 5**
Ht	0.213896	0.679309	0.415761	-0.404117	0.395752
LAI	0.621603	-0.092075	0.325613	-0.163776	-0.68723
Salt1	-0.09663	-0.67401	0.607059	-0.223023	0.343677
Salt2	-0.47314	0.271989	0.588503	0.511193	-0.307398
Till	0.578486	-0.04236	0.079139	0.706264	0.398104

Prin1, Prin1, Prin2, Prin3, Prin4 and Prin5 = Principal Component Analysis 1, 2, 3, 4 and 5, respectively.
Ht = plant height, LAI = leave area index, Till = number of tillers, Salt1 = first salinity score and Salt2 = second salinity.

3.3. Phenotypic Correlation Among Traits

The number of tillers both at the first and second salinity scores had a significance and negatively correlated at *(P ≤ 0.05*) and (*P ≤ 0.01)*, respectively and number of tillers positively correlated with plant height and LAI (Table 7).

Table 7. *Control Soil Experiment Correlation Matrix.*

	Ht	LAI	Salt1	Salt2
Ht				
LAI	0.446**			
Salt1	0.4419**	0.1406ns		
Salt2	0.0118ns	-0.156ns	0.5624**	
Till	0.3517*	0.7619***	-0.2458*	-0.495**

*, **, and *** significant at 0.05, 0.01 and 0.001 probability level respectively, ns = non-significant

In the coastal soil experiment, the number of tillers and LAI at the second salinity score negatively correlated with a significance difference at *(P ≤ 0.05*) and (*P ≤ 0.01)*, respectively The LAI had a significance correlation with number of tillers at (*P ≤ 0.01)* Table 8.

Table 8. *Coastal Soil Experiment Correlation Matrix.*

	Ht	LAI	Salt1	Salt2
Ht				
LAI	0.239ns			
Salt1	-0.358*	0.081ns		
Salt2	0.094ns	-0.429**	0.031ns	
Till	0.101ns	0.565**	-0.083ns	-0.323*

* and ** significant at 0.05 and 0.01 probability level respectively, ns = non-significant

4. Discussion

Most of the genotypes tested showed improved tolerant to salinity as plants advance in age in the two experiements, indicating some physiological adjustment to K+ / Na+ imbalance, this corroborated the earlier report Devitte et al., [15]. The severity of salinity effect was observed to be higher in coastal soil experiment base on reduction of all traits measured as compared to the resistant check (Pokkali), particularly, there was high reduction in leaf area index and plant height this corroborate with report Gregorio et al., [11]. Emoghene et al. [21] reported similer observation on reduction in germination percentage and relative dry weight (% RDW) of seedlings. In the coastal soil experiement, differential response of genotypes to salinity stress were observed based on their agronomic performance, similar observation were reported Egnard et al., [22], Jampeetong and Brix, [6] and Munns and Tester [7].

The t-tesst values showed that the two experiemnts were significantly diferent based on salinity sevrerity and genotypes response to salinity stress. This indicates the genotypes performance in the coastal soil experiement are not by chance, this could be regarded as a good screening protocol for salinity. High leaf area index (LAI) is likely to be more efficient of photosynthetic process contributing to high grain yield, Efisue et al. [5], reported high LAI of rice under drought stressed, which indicated that LAI is a good selection criteria for high grain yield. Therefore, genotypes with higher value of LAI could be high yielding, such as IR 84105-5-B-1-B-3 (3.40) and IR 84105-5-B-1-B-2 (3.03).

Tillering ability is one of the most import yield components of rice, which determines higher grain yield. Few tillers could result to too few panicle formation, conversely, excess tillers may result to high unproductive tillers and poor grain yield Peng et al., [23]. Therefore, an appreciable number of tillers per plant may translate to effective panicle production, genotypes such as IR 84105-5-B-1-B-1 (15), IR 84105-5-B-1-B-3 (15) and IR 84105-5-B-1-B-2 (12) could be promising.

The salinity scores showed high significant negative association with tiller number and leaf area index. These associations indicate that increase in salinity stress will reduce the tillering ability and the leaf area index. These aforementioned traits are very important for high grain yield in rice Efisue et al., [12]. In principal component 1 (Prin 1), the leave area index and tiller number per plant had high weight and factor loadings as compered to other trits observed, thus indicate Prin 1 reprensents a laten variable for physiological taits, while Prin 2, plant height had high weight and factor loading, thus implied that Prin 2 represnets latent variables for morphological traits as explained by Iezzoni and Pritts [24].

5. Conclusion

Salinity stress affect the productivity of rice in saline enivironments. Developing genotypes with inherent salinity tolerant could be economical and cost effective. The protocol used in this study could be recommeneded for effective salinity screening experiemt. Genotypes IR 84105-5-B-1-B-3 and IR 84105-5-B-1-B-2 maybe promising for deployment into this salinity stressed region. The leave area index and tillering ability of the rice crop are major yield components, their high weight and factor loading values indicate that they are purtative traits for salinity tolerant, which could be used for population development in salinity breeding programme.

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