Journal of Genetics, Genomics & Plant Breeding **4(3) 147-156 (July, 2020)**
ISSN (Online): 2581-3293

RESEARCH ARTICLE

Comparative analysis of rice genotypes for tolerance to salt stress at the seedling stage

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Manuscript received: June 4, 2020; Decision on manuscript, July 7, 2020; Manuscript accepted: July17, 2020

Abstract

Different and complementary sources of salt tolerance are necessary for developing tolerant varieties. A newly identified rice variety tolerant to salt stress was compared with known checks (FL478- tolerant and IR29-susceptible) and BG90-2 (mega West African variety) under hydroponic conditions at 12dSm^{-1}. The four varieties were assessed using six growth traits; shoot height, leaf number, shoot dry weight, root number, root length and root dry weight and salinity tolerance score. Significant variation in salt tolerance traits were observed at $p<0.05$. Salt injury score for the varieties were as follows: FL478 (7.23) Jarmissa (7.42) BG90-2 (7.67) and IR29 (9.0). The first axis of the PCA associated more with shoot dry weight and explained 98.99 % variation. Three groups were formed at 55% dissimilarity as follows: IR29 in group 1, FL478 in group 2 and Bg90-2 and Jarmissa in group 3. Shoot dry weight coupled with increased root length provides better ability to withstand salt stress. Hence, researchers could use shoot dry weight as proxy to indirectly estimate salt tolerance in rice germplasm for breeding and other research purposes. Jarmissa shares similar properties and reactivity to salt stress with the Mega West African rice variety BG90-2, thus it could be used directly in breeding programme or adapted to less demanding environments.

Key words: Rice, salt tolerance, seedling stage, hydroponics, PCA

Introduction

Soil salinity is the second most devastating constraint in rice production after drought, affecting approximately 1 billion ha of land globally (Fageria *et al*., 2012, Bimpong *et al*., 2014). Salinity concerns are increasing in rice (*Oryza sativa* L.) fields in Sahelian West Africa, where reduced precipitation and high temperatures conditions causes high evapo-transpiration (Ndour *et al*., 2016; Goita, 2015; Sylla, 1997; Heenan *et al*., 1988). Modelling climate change impacts in West Africa it has been reveale that the region is approximately 1 °C warmer with fluctuating wet and dry periods, which have implications for crop yield (Roudier *et al*., 2016; Sultan and Gaetani, 2016). High levels of salinity affects rice growth to varying degrees at all stages starting from germination to seed maturityin rice (Vispo *et al*., 2014; Aliyu *et al*., 2010; Kumar *et al*., 2009; Manneh, 2004).

The most vulnerable growth stages of rice to salt stress are early seedling and reproductive stages (Ismail, 2007). Salt stress at the seedling stage induces premature senescence of leaves, reduction in plant height, leaf production and dry matter production (Munn and Tester 2008, Dudhe and Kumar, 2016). Therefore, productivity in salt affected areas can significantly be improved using salt tolerant varieties (Seck *et al*., 2013). Most of the popular varieties of rice grown in West Africa are susceptible to salt stress and genetic resources for salt tolerance development are limited. The effects of salinity on plants are complex and easily modulated by environmental conditions (Shannon, 1997). Salt tolerance is known to be a multi-genic trait expressed by several parameters including morphological Physiological and biochemical parameters (Kakar, *et al*., 2019; Ismail and Horie, 2017;Samant and Jawali, 2016; Chunthaburee *et al*., 2015; Barua *et al*., 2015 Negrão *et al*., 2013; Abbas *et al*., 2013; Razzaque *et al*, 2009).The decline in growth parameters under saline conditions have been reported in different plants under salt (Kumar *et al*., 2009; Ali *et al*., 2014) and therefore comparison of plant growth under stress and non-stress conditions serves as the basis for identifying tolerant lines. Principal component Analysis (PCA) is an inter-relational statistical technique that explains large number of variables with reduced set of variables. The new set of variables, the principal components (PCs), is uncorrelated and the first few retain most of the variation (Jolliffe, 2002, Negrão *et al*., 2017; Kumar *et al*., 2018). The statistic is useful for the compression and classification of data. According to Shimilis *et al*., (2013), PCA helps breeders to consider a few important variables and reduces the cost of screening in breeding programmes. In this study, Jarmissa, a newly identified germplasm, tolerant to salt stress was compared with known international checks (FL478 and IR29) and West African Mega variety (BG90-2). The objectives were to confirm salt tolerance expressed by Jarmissa under natural growing conditions and to identify the most important morphological descriptors of salinity tolerance at early growth stage

Materials and methods

Four rice varieties (Jarmissa, IR29, BG90-2 and FL478) were used in this study. Jarmissa is a germplasm identified in the Casamance region in The Gambia, IR29 (salt susceptible check), BG90-2 (high yielding variety in irrigated parameters of West Africa) and FL478 (salt tolerant check) were all obtained from Africa Rice Sahel Regional Station, Saint Louis, Senegal. Pre-germinated seeds were sown in holes on Styrofoam seed try suspended in Yoshida nutrient solution (Yoshida *et al*. 1976). Salt stress was imposed 5 days after germination by adding crude salt (sodium chloride) first to an electrical conductivity (EC) of 6 dSm^{-1} and then to 12 dSm^{-1} in Yoshida nutrient solution until final scoring. The EC and pH of the nutrient solution were adjusted to 5.0 and $12dSm^{-1}$ three times a week. The culture solutions were replaced every 8days. The experiment was replicated 18 times with five individuals per replication. Temperature and humidity figures during the experimental periods were estimated using data loggers. Performance of the four varieties were assessed based on visual symptoms using Standard Evaluating Score (SES) for salt tolerance, on a scale of 1-9, (1 - highly tolerant and 9 - highly susceptible) and six agro-morphological traits: Leaf number, shoot height, shoot dry weight, root number, root length and root dry weight.

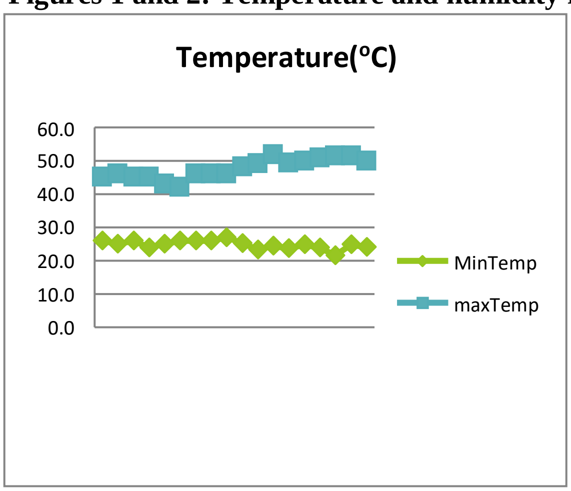
Seedlings were scored 1 if they showed no symptom of stress, 3 when seedlings showed little to no leaf damage, but was stunted compared to the corresponding genotype grown in the control solution, 5 when seedlings was stunted with green rolled leaves having a few whitish tips, 7 when only green culm with dried leaves and 9 when seedlings were completely dead. Visual scoring was done after 21 days after stress application. Root length and shoot height parameters were measured with a rule whereas dry weight parameters were taken after samples were dried at 50^{o}C for 3 days. Tolerance to salt stress was estimated using the salt tolerance index. Differences between accessions were assessed by analysis of variance of trait values after which principal component analysis and Hierarchical clustering was performed using R statistical package.

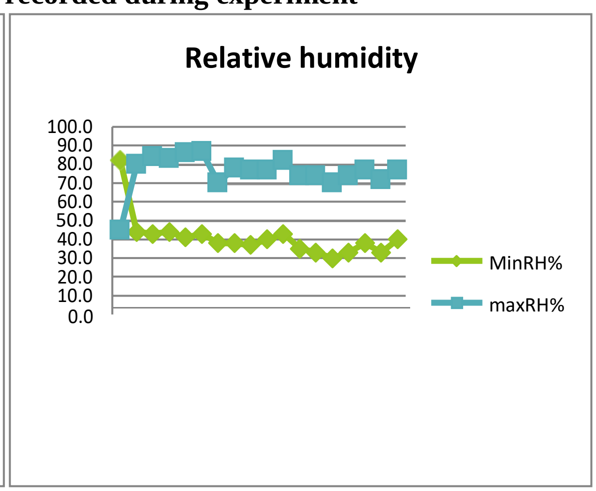
Results and discussion

Environmental conditions

Experiment was carried out in Ndiaye, near Saint Louis, Senegal (16^{o} 14'N, 16^{o} 14'W). The minimum and maximum temperatures conditions ranged between 21 - 25^{o}C and 42 - 52^{o}C (Figure 1) and the minimum and maximum relative humidity values ranged from 30- 43 and 70.0 and 88 (Figure 2). The observed high fluctuating temperature and humidity observed during the experimental period, is typical of Sahelien environments. The climate conditions (Figure 1 and 2) reported were similar to conditions observed by Ndour *et al*., 2016. However, the temperature and humidity differed from values reported by Asch *et al*. (1997) for this same location during the wet season of 1997. Mean maximum temperature values for instance were up from 42^{o}C to 52^{o}C. The increase in temperature could increase evapo- transpiration rates and enhanced saline conditions. Sultan *et al*., (2019) used two crop models (SARRA-H model and CYGMA model) have shown evidence of crop losses due to frequent heat and rainfall extremes in West Africa. It is therefore important to identify new adapted varieties if we are to maintain current levels of rice production in the region. Generally, for all traits studied, salt stress caused reduction in performance compared to the control (Fig 3a-f). This observation agrees with report by Razzaque *et al*., (2009) in which they found an adverse effect of salt on plant height, root, shoot and dry matter of seven rice genotypes.

Figures 1 and 2: Temperature and humidity recorded during experiment





Analysis of variance (Table 2) indicated that environment was highly significant for all measured traits. The difference found between varieties for leaf number in the two environment was significant at $P<0.05$ and the differences between varieties for the five other traits were significant at $p<0.01$.

Genotype by environment interactions was not significant for shoot height and root number, significant for leaf number and root dry weight and highly significant for root length and shoot dry weight (Table 2). Earlier researchers have reported that shoot length, root length and plant biomass are effective salinity tolerance indicators (Gregorio and Senadhira 1993; Zeng *et al*., 2007; Munn and Tester 2008; Kumar *et al*., 2009; Ali *et al*., 2014; Dudhe and Kumar, 2016). In all the six traits evaluated, performance of genotypes followed the order: FL478, Jarmissa, BG90-2 and then IR29 (Table 3). Similar trend was observed in the salinity score data. The relationship between genotype and environment (Fig 3a-f) were generally linear, which, implied that genotypes performing better under controlled conditions necessarily performed better under stressed condition. In the case of FL478 root length increased under salt stress (Table 3). This rare occurrence possibly gave FL478 a better potential to withstand salt stress than the three other varieties. The better performance exhibited by FL478 in the other traits could be attributed to the increased root length under stress condition, which permitted the absorption of more nutrients. Past studies have shown that salinity retards plant growth mainly by affecting root development and growth, which, helps tolerant genotypes to maintain vigorous shoot growth (Barua *et al*., 2015, Lui *et al*., 2019). According to Flowers and Yeo (1988), a deficiency of nutrient and assimilate supply for developing organs in salt stress conditions contributed to reduced growth and eventual plant death in susceptible genotypes.

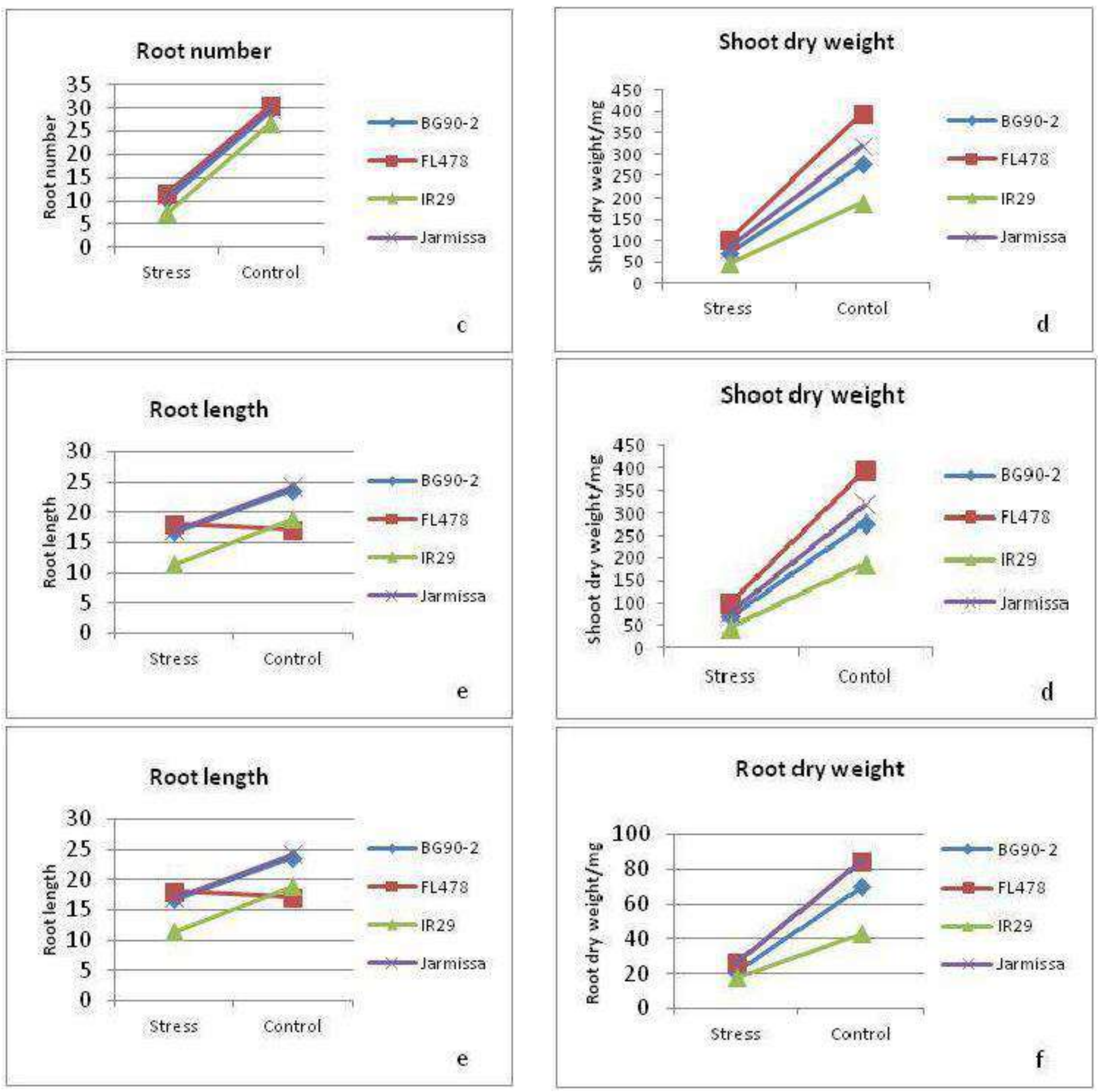
In the current study, Principal Component Analysis (PCA) revealed that shoot, and then root parameters contributed to most of the variation observe. The first principal component (PC1) explained 98.99 percent of variation among the study genotypes (Table 4). Variation at the first principal component axes (PC1) was attributed to two traits: Shoot dry weight (97%) and root dry weight (22%). Four out of the six parameters (height, root number, root dry weight and shoot dry weight) showed inverse relationship with PC1, indicating parameters were reduce by the salt stress imposed. The second principal component (PC2) explained 0.99% of total variation. 87% of this variation is attributed to root dry weight and 39% to root length. In an independent study, Kakar and colleagues found the first two principal components (PCs) accounting for 45% and 13% of the total variation (58%) among rice genotypes (Kakar *et al*., 2019). The results generally agrees with several other authors who reported that shoot dry weight is a useful criteria for screening for salt tolerance in rice (Jamil *et al*., 2007; Akbar *et al*., 1986; Ali *et al*., 2004). Shoot dry weight is therefore the most useful traits for distinguishing the four varieties.

From the cluster analysis (Figure 5) Jarmissa clustered with BG90-2 at about 25% dissimilarity coefficient. However, Jarmissa was joined to FL478 at height 110 where there is approximately 55% dissimilarity. IR29 clustered away from the remaining three varieties at height 170. This observation confirm the results based on standard evaluation score (Table 3). Jarmissa shared similarity with varieties BG90-2, 2 FL478 and IR29 in descending order of closeness

In the current study FL478 was considered to be moderately susceptible to salt stress in contrast to earlier reports (Gregorio *et al*., 1997; Walia *et al*., 2005) which concluded that FL478 was tolerant to salt stress. Dingkuhn *et al*., (1994) had earlier indicated that varieties like Pokkali and Nona Bokra which tested salt tolerant under Asian monsoon conditions showed susceptibility under arid conditions of the Sahel.

(1997) explained that varieties which did not accumulate sodium to toxic concentration under monsoon conditions were likely to accumulate lethal doses of sodium under Sahelien conditions because of low humidity and increased evapo- transpiration in the Sahel.

Figures 3 (a-f): Performance of rice varieties under salt stress and non-stress conditions: a - shoot height, b- leaf number, c- root number, d -shoot dry weight , e -Root length and f - Root dry weight



De Leon *et al*., (2015) had indicated the difficulty in attaining consistency and reproducibility of results between laboratories worldwide using one screening scale for determining salinity tolerance, due to non- uniform growth environments. It is been observed that climate modulate the physiological effect of salt on plants and the initial impact of salt stress is due to water deficit (Munn and Tester, (2008). Therefore it will be inappropriate to use the same scale for rating varieties under different climatic conditions. The different factors in the balance of water: temperature, intensity of precipitation, evapotranspiration rate and humidity conditions ought to be considered in rating species for salt tolerance.

Meanwhile since FL478 was rated tolerant in studies conducted elsewhere, it is possible that Jarmissa could also be rated tolerant under similar environment because no significant difference was found between the salt injury score for the two varieties although FL478 showed superior performance in all the measured traits except leaf number (Table 1).

Table1: Mean trait value recorded in four varieties at under stressed and non-stressed conditions, 21 days after stress application

Environment	Variety	Leaf number	Shoot height,	Root number	Root length	Root dry weight	Shoot dry weight
Stress	Bg90-2	5.39	19.64	10.26	16.64	19.40	68.80
Stress	FL478	5.61	27.05	11.52	17.82	25.20	95.90
Stress	IR29	4.07	16.09	7.55	11.39	16.00	40.60
Stress	Jarmissa	5.76	21.10	11.36	16.83	23.40	75.50
non-stress	Bg90-2	8.03	45.42	29.46	23.60	68.90	272.70
non-stress	FL478	8.75	52.61	30.59	17.14	85.60	402.20
non-stress	IR29	7.94	41.96	26.80	19.04	43.80	191.00
non-stress	Jarmissa	9.17	46.08	29.99	24.34	86.10	323.30
SE.		1.60	6.82	3.80	3.32	20.49	77.39
CV%		23.40	20.20	19.30	18.10	44.40	42.00

Table 2: Analysis for variance various growths attributes under salt stress (12dSm-1) and non-stress (0.15dSm-1) conditions

Source of variation	d.f.	Leaf number	Shoot Height	Root Number	Root dry weight	Root length	Shoot dry weight
Block	17						
Genotype	3	695.07*	695.07**	92.96**	4928.8**	208.69**	107557**
Environment	1	23141.05**	23141.05**	12858.04**	89662**	1009.51**	1840330**
Gen. Env	3	4.52*	4.52^{ns}	2.43^{ns}	2259.8*	146.24**	39052**
Residual	134	46.46	46.46	14.42	419.9	11	5990
Total	141						

**= highly significant, *= Significant, ns = not significant Gen =genotype. Env = environment

Table 3: Percentage reduction ROC under salt stressed compared to non-stressed conditions

Variety	Leaf number (%)	Shoot height (%)	Root number (%)	Root dry weight (%)	Shoot dry weight (%)	Root dry weight (%)	Reaction to salinity
FL478	35.09	50.57	61.37	-2.00	74.77	69.36	Sensitive (7.23)
Jarmissa	36.44	54.42	61.66	30.46	75.39	71.36	Sensitive (7.42)
Bg90-2	34.18	56.37	64.26	29.54	74.53	68.43	Sensitive (7.67)
IR29	45.19	60.28	70.07	39.01	75.19	55.41	Highly sensitive (9.0)

Table 4: Principal component analysis of six morphological traits

Characters	PC1	PC2	PC3	PC4
Leaf number	0.00	0.03	-0.20	-0.98
Plant height	-0.06	-0.25	0.29	-0.07
Root number	-0.02	0.05	0.35	-0.08
Root length	0.01	0.39	0.82	-0.15
Shoot dry weight	-0.97	-0.18	0.05	-0.15
Root dry weight	-0.22	0.87	-0.29	0.08
Proportion of variance	0.9899	0.00998	0.00011	0.00E+00
Cumulative proportion	0.9899	0.99989	1	1.00E+00

Figure 4: A genotype by trait biplot based on principal component score

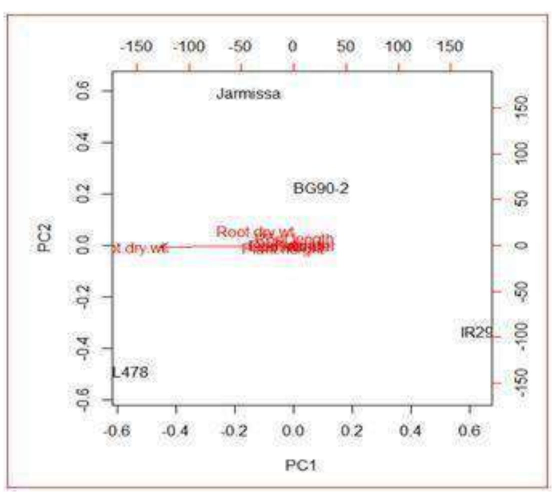
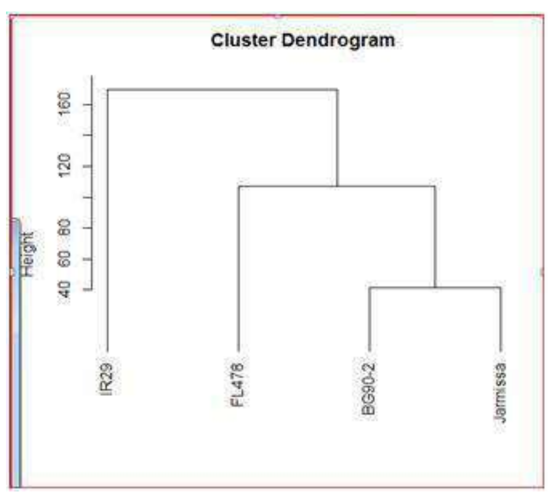


Figure 5: Phenogram of four rice varieties on salt tolerance parameters



Conclusion

The landrace, Jarmissa expressed similar tolerance as FL478 but clustered together with Bg90-2. The study identified that shoot dry weight parameter was better predictors of salinity tolerance and can be used by rice breeders and other scientists to screen and select salinity tolerant rice lines for variety development and related research. Further detailed studies may be necessary to elucidate mechanism of salt tolerance in Jarmissa. Considering the recent fluctuations in weather patterns, and the fact that rice plants may be subject to salt stress at any development stage, it may also be useful to screen Jarmissa for salt tolerance at the other growth stages.

Acknowledgements

The authors gratefully thank AfricaRice, Sahel Regional Station for supporting Research and Alliance for Green Revolution in Africa (AGRA) for funding travel cost.

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