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Phenotypic Responses of Oryza Species to Saline Condition at Reproductive Growth Stage

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: This research aimed at evaluating the response and morphological effects of salt stress on Oryza species at vegetative and reproductive growth stage.

Study Design: Salt tolerance was evaluated by adopting the Standard Evaluation System of IRRI for salt tolerance under modified hydroponic systems.

Place and Duration of Study: The investigations for this study were conducted at AfricaRice Station at the International Institute of Tropical Agriculture (IITA), Ibadan (Latitude 354¹N and longitude 7'30¹W), Nigeria and the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria.

Methodology: Forty rice (Oryza sativa (20), Oryza glaberrima (10) Oryza barthii (05) and NERICA (05) genotypes encompassing 20 tolerant and 20 susceptible pre-screened genotypes to salinity stress at seedling growth stage were subjected to salinity stress at early vegetative growth stage. The sensitive (IR29) and tolerant (POKKALI) checks served as controls for susceptibility and tolerance respectively. These genotypes were subjected to salinization with NaCL at EC 8dsm⁻¹ at pH 5.2 till maturity. Plant phenotypic responses were evaluated to ascertain specie response. **Results:** Results acknowledged that the effect of salinity on plant growth was genotype and specie

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dependent. The interactions between genotypes and traits evaluated were highly significant (P< 0.01). Tolerance at seedling stage did not culminate to tolerance at reproductive stage. Phenotypic response to salinity stress at reproductive stages showed strong (p<0.01) negative association between salinity evaluation score (SES) to plant height (r^2 = -0.5), culm length (r^2 = -0.5) and filled grain (r^2 = -0.5). Salinity stress adversely affected panicle emergence and caused aborted spikelet, thus suppressing rice yield. The grain length of susceptible genotypes increased significantly. A 55% increase in brown rice shape was obtained. Tolerance range for survival at reproductive stage for Oryza sativa and Oryza glaberrima were 90 and 40% respectively. Oryza barthii and NERICA were most susceptible to salt stress and failed to set seed at reproductive stage. Six (15%) genotypes showed tolerance comparable to the tolerant check at maturity while 7 (17.5%) genotypes were moderately tolerant to salinity. Six susceptible genotypes (15%) with an SES score of 7 at reproductive stage set seed.

Conclusion: The effect of salinity stress on plant growth and yield were genotype and specie dependent. Salinity adversely resulted in reductions in plant biometrics. Degrees of growth plasticity were observed in some genotypes as an escape strategy against salinity. Salt stress induced changes in grain lengths and seed shape. The presence of flag leaf and penultimate leaf or few leaves before panicle initiation determind the genotype ability to set seed at reproductive growth stage.

Keywords: Salt injury; salt tolerance; Oryza sativa; Oryza glaberrima; Oryza barthii; NERICA; reproductive stage.

1. INTRODUCTION

Water-deficit and salt affected soil are two major abiotic stresses which reduce crop productivity, especially that of rice by more than 50% worldwide [1,2]. Salt stress responses and tolerance vary between species [3,4]. The limiting concentration changes with plant species, variety and stage of development and duration of the salt stress [5]. The capacity to tolerate salinity is a key factor in plant productivity [6]. Salt tolerance is the ability of plants to grow and complete their life cycle on a substrate that contains high concentrations of soluble salt [7]. Salinity associated with excess NaCl adversely affects the growth and yield of plants by depressing the uptake of water and minerals and normal metabolism [8,9]. The intercellular water potential is thereby lowered below the external water potential allowing continued water uptake. Rice is one of the most important crops that provide food for about half of the world population with its adoption as a principal staple food is increasing in Africa [10]. The importance of this crop is progressively recognized for its nutritional value and because it is an integral part of religious and social ceremonies. Rice is a key source of food energy and rice-based production and processing are major employers and source of income for the poor [11]. In all major rice growing countries, the rice-land farming systems involving crops, livestock and fish farming continues to sustain agricultural infrastructure and many associated value-adding rural raw materials needed by the manufacturing industry. Thus poor harvest has an adverse effect on many nations' economies [12]. Meeting the challenges of sustainable increases in rice production and production efficiency is thus vital not only for food security but also for alleviation of poverty of several hundred million farmers in low-income and developing countries. However, approximately half of the world's land surface is a perennial desert or dry land (United Nations Development Programme). These can be made more productive by irrigation which is unfortunately strongly linked to salinization as earth is a salty planet, with most of its water containing about 30g of NaCl per litre. This salt solution has affected and continues to affect the land on which crops are or might be grown [11,13]. Rice production in Africa increased from 8.6 million tonnes of paddy in 1980 to 18.6 tonnes in 2005. Despite such dramatic growth, demand continues to exceed supply and the region relies on importing rice [14]. The increasing population has thus converted highly productive rice lands for industrial and residential purposes and has pushed rice cultivation to less productive lands including salinity prone areas. To meet the projected growth in the demand for rice as income increases, rice production must increase to 758 million tones by 2020 [15]. The immense potential of the lowland which offers great sustainable expansion and intensification of rice to feed the growing population in West and Central Africa have not been realized due to

enterprises and services as well as providing the

biotic and abiotic constraints [14]. The crop grown under extensive irrigation regimes is unusually susceptible to salinity stress [16] as soil salinity is a major problem in modern agriculture particularly for irrigated croplands [17].

The ever increasing population demands the intensive as well as extensive agricultural activities of crops especially to improve their productivity in problem soils. Selection of highly salt tolerant genotypes within and between species can be expected to provide useful materials [18] to develop salt tolerant varieties as there exist tremendous variations for salt tolerance within species in rice. Therefore, this research is aimed at evaluating the responses and morphological effects of salinity stress to Oryza species at reproductive growth stage as there is a great deal of urgency for developing rice genotypes which can sustain and set seed under high salt stress conditions to enhace productivity.

2. METHODOLOGY

2.1 Evaluating Rice Genotypes for Salinity Tolerance at Reproductive Stage

Forty rice (Oryza sativa (20), Oryza glaberrima (10) Oryza barthii (05) and NERICA (05) genotypes encompassing 20 tolerant and 20 susceptible pre-screened genotypes to salinity stress at seedling growth stage were subjected to salinity stress at the late seedling growth stage. The sensitive (IR29) and tolerant (POKKALI) checks served as controls for susceptibility and tolerance respectively.

2.2 Screening for Salt Tolerance at Reproductive Stage

Holes of about 3-4 mm and 2cm apart were drilled into the walls of plastic pots (25 x15cm) to about 5cm to the top of the pot. Jute bags were filled with sterilized soil and placed inside the plastic pot. Fertilizer (N.P.K-20-20-20) was applied to the soil at the rate of 5kg/ha. The soil was filled 1cm above the topmost circles of holes in the pot. The pots were placed in large plastic trays filled with tap water to the same level of the soil. Each plastic tray contained five pots. Eight plastic trays were used per replicate. Three replicates were laid in a complete randomised

design in the screen house. Rice seeds were cleaned and placed in an oven for 3-5 days at 30°C to break seed dormancy. The seeds were surface sterilized with 1:5 benlate solutions. Sterilized seeds were sown directly on soil at a seed rate of 4 per pot at a depth of 1 cm below the soil surface. Two weeks after seeding, seedlings were thinned to 2 plants per pot. The water level in the tray was raised to 1 cm above soil level and this was maintained daily.

Twenty-one days after seeding, the water in the plastic trays was siphoned. 12 hrs later, the water dripping from the plastic pots were also siphoned. Salt solutions of EC 8dsm⁻¹ was prepared and used to fill the plastic trays till it was 1 cm above soil level to induce salinity stress. The water level in the plastic tray was maintained daily with tap water. The non-saline control treatment was composed of Peter's 20- 20-20 water soluble fertilizer, ferrous sulphate (0.1 g/l) and tap water.

2.3 Phenotypic Evaluation at Reproductive Stage

Growth and yield evaluations were as documented for the salinity evaluation score for Rice (IRRI, 1997)

(http://www.knowledgebankirri.org/ses/SES.htm) as follows; Tiller number per plant (TN), Plant height (PH) in cm, Panicle length (PL) in cm, Spikelet (SPKT) Filled Grains (Fg), Panicle (P) per plant, unfilled grain (Ug) per panicle, days to maturity (DM), Culm length (CML) in cm, Lodging (L), Leaf width (LW) in cm, Grain length (Len) in mm, Grain width (GW) in mm, Grain length to width ratio (GL/GW), 100-grain weight (g), Root length (RL) in cm, Shoot fresh weight (SFW) in mg, Root fresh weight (RFW) measured in mg, Shoot dry weight (SDW) measured as the weight (in mg), Root dry weight (RDW) in mg, Ph/Rl, Shoot fresh weight/root fresh weight (SFW/RFW), Root dry weight/Shoot dry weight (RDW/SDW).

2.4 Statistical Analysis

Analysis of variance (ANOVA) was used to compare means. Where significant, mean were separated by ranking using Duncan's Multiple Range Test (DMRT) for each parameter. These were performed using the GLM procedure of Statistical Analysis System [19]. The correlations between morphological characters were

analyzed simultaneously by stepwise analysis [20].

3. RESULTS AND DISCUSSION

The effect of salinity on plant growth was genotype dependent. Plants under control treatment showed normal growth with no symptoms of salt injury. However, plants under salt stress were severely (p>0.01) affected by salinity symptoms. At reproductive stage, 14 genotypes exhibited some degree of tolerance to salinity (SES=1-5) while 26 genotypes showed susceptibility (Fig. 1). Eleven (78%) of the tolerant genotypes at reproductive stage showed tolerance at seedling stage; additionally 3 tolerant genotypes (BW 294-57, WITA 4 and CK 73) showed tolerance but were susceptibility at seedling stage. About 45% of the tolerant genotypes at seedling stage exhibited susceptibility at reproductive growth stage while 15% of susceptible genotypes at seedling stage showed tolerance at reproductive stage.

Between species, susceptibility were recorded at 20, 100, 100 and 60% for Oryza sativa, O. barthii, O. glaberrima and NERICA at respectively (Fig. 2).

About 50% of the genotypes with SES score of 1 to 7 survived till maturity. Six (15%) genotypes showed tolerance comparable to the tolerant check at maturity while 7 (17.5%) genotypes were moderately tolerant to salinity. Six susceptible genotypes (15%) with an SES score of 7 at reproductive stage set seed.

Fig. 1. Salinity evaluation score for 40 genotypes at reproductive stage Key: 1-9 are tolerance levels where 1>2>3>4>5>6>7>8>9 1-3: Tolerant; 4-6: Moderately tolerant; 7-9: Susceptible

3.1 Plant Height and Lodging

Plant heights and lodging varied significantly across genotypes (Table 1). A negative correlation between plant height and SES was observed. Plant heights were significantly (P<0.05) shorter under salt stress, with reductions in height of about 22.4% and 29% in Oryza sativa and Oryza glaberrima respectively (Fig. 2). Reduction in plant height for POKKALI was 23%.

Salt stress significant (p<0.01) induced lodging. The average effect of lodging between species was most pronounced in Oryza sativa genotypes (40%) compared to Oryza glaberrima genotyoes (33%) (Fig. 2).

Fig. 2. Salinity evaluation score at vegetative growth stage

Table 1a. Growth parameters on characterized traits at reproductive stage

Table 1b. Growth parameters on characterized traits at reproductive stage

3.2 Days to Maturity

Days to maturity amongst genotypes varied significantly (Table 1). Seventeen (85%) genotypes showed early maturity under salt stress. These genotypes matured earlier under stress than in controlled conditions. The earliest maturing genotype under salt stress was SIPI 692033 (82 days) (Table 1). All Oryza glaberrima genotypes under salt stress matured 3 days earlier than unsalinized treatment. In Oryza sativa 3 genotypes matured later than the unsalinized treatment. Reductions in days to maturity ranged from 2 to 40 days. Average reductions in days to maturity recorded for O. glaberrima and Oryza sativa genotypes were 3% and 11.20% respectively (Fig. 2).

3.3 Culm Length and Panicle Length

Culm and panicle lengths varied significantly (p<0.01) amongst genotpes. Oryza glaberrima and Oryza sativa genotypes showed an average increase in culm length of 2% and 9.1% respectively (Fig. 2). Approximately 25% of genotypes evaluated at maturity showed increase in panicle length ranging from 5 cm to 16 cm in Oryza sativa and as high as 21 cm in Oryza glaberrima (Table 1). General reduction in panicle length was 8.22%.

3.4 Leaf Length and Leaf Width

The leaf length and width of all genotypes significantly decreased under saline conditions. Some degree of growth plasticity was observed where 15 and 20% of the genotypes exhibited an increase in leaf length and width respectively. Oryza glaberrima genotypes showed lower reductions in leaf length and width than O. sativa genotypes. This was noted by the average percentage reductions in leaf lengths and width of 15.6% and 16.7% in O. sativa compared to the average of 14.3% and 9.1% obtained in O. glaberrima (Fig. 2).

3.5 Culm Number

Salt stress adversely affected culm number. About 25% of the genotypes showed increase in culm number ranging from 20 to 66%. The culm number of 15% of the genotypes was not affected by salt stress. An average reduction of 14.3 and 16.6% in culm number for Oryza glaberrima and O. sativa genotypes was obtained respectively (Fig. 2).

3.6 100-Grains Weight

One hundred well developed whole grains dried to a moisture content of 13% for all genotypes showed varied but significant (p<0.01) response to salt stress (Table 1). The grain weights of 30% of the genotype were no affected by salt stress. Grain weight (2.56 g) recorded was highest in and lowest in ARG6625 (1.40 g) (Table 1). Oryza glaberrima showed an average reduction of 15.4% in grain weight while, O. sativa showed a 9.8% reduction (Fig. 2).

3.7 Grain Length (GL) and Grain Width (GW)

Reduction in grain length also ranged from1% in IR 77660-3B-29-1-2-2-13 to 20% in CK 73, FARO 19 and PSB Rc50. The Grain lengths significantly (p<0.1) increased in most of the susceptible genotypes. The grain length of SIPI 692033 (9.23 mm), BW 294-5, ARG 6625 and ITA 306, increased by 12%, 2%, 9% and 10% respectively under salt stress.. POKKALI showed a 12% decrease in grain length. Oryza glaberrima genotypes showed an 8% increase in grain length while O. sativa showed an 8% reduction in grain length (Fig. 2). The distance across the fertile lemma and the palea at the widest point of all genotypes varied considerably and was significant (p<0.01) in all genotypes. The average grain width of O. glaberrima genotypes was 2.56 mm. Oryza glaberrima with an average grain width percent reduction of 12.7% was not comparable to O. sativa genotypes that showed no grain reduction (Fig. 2).

3.8 Brown Rice Length (LEN) and Brown Rice Width (BRW)

Brown rice length did not increase in O. glaberrima genotypes. However, significant increase of 8.1% was notable in few O. sativa genotypes (IR 77660-3B-29-1-2-2-B, SAHEL 108 and BG 2765). Other genotypes showed reductions in length ranging from 2% in FL478 to 19% in POKKALI and TOX 4004-3-1-2-1 (Table 1). Greater reduction in brown rice length was more pronounced in susceptible than tolerant genotypes. Average reduction in brown rice length was 8.1% (Fig. 2).

Brown Rice Width varied significantly in all genotypes and was narrowest in WITA 4 (1.72 mm) and widest in PSB Rc50 (2.33 mm), BG

276-5 (2.33 mm) and FL 478 (2.32 mm). Brown rice width in POKKALI was 2.17 mm (Table 1) with a percentage reduction of 21% under salt stress. Reductions were not observed 20% of the genotypes. The average percentage reduction of 27% in brown rice width of O. glaberrima was most pronounced compared to the average of 1.3% reductions in O. sativa (Fig. 2).

3.9 Brown Rice Shape (BRS)

This is a ratio of the brown rice length (len) to width (brw). Brown rice shape was significantly affected in all genotypes under salt stress (Table 1). Salinity stress however did not change the shape of POKKALI and BG 2796. About 55% of the genotypes showed increase in brown rice shape ranging from 3% in TOX 4004-3-1-2-1 to 32% in TOG 5601 and TOG7428. A decrease in brown rice shape ranged from 3% in SAHEL 108, Bouake 189 and CK 73 to 12% in PSB Rc50. Oryza glaberrima genotypes generally showed a higher percentage increase (30.7%) in BRS than O. sativa genotypes (5.1%) (Fig. 2). The slender grained FL478 and IET 3137 became medium shaped genotypes under salt stress. Medium grained ITA 306, TOX 4004-3-1-2-1, FARO 19, ARG 6625 and IR 77660-3B -29-1-2-2-B became slender grained rice genotypes under salinity stress. 25% of the genotypes that were initially medium shaped became slender and 10% of slender grained genotypes became medium shaped. The shapes of O. glaberrima was not affected but tended towards slender grains.

3.10 Filled and Unfilled Grain

The number of filled grains was negatively associated (r=-0.5) with SES and decreased significantly (p<0.1) across genotypes. Unfilled grains ranged from 6% in SIPI 692033 to 77% in TOG 5601. The reduction in percentage filled grains in POKKALI decreased by 39%. Susceptible genotypes under salt stress recorded the least number of filled grains and highest unfilled grain percentages. Oryza glaberrima recorded the lowest percentage of filled grains (33.9%) under salinity stress while, O. sativa had 53.6% filled grains (Fig. 2).

3.11 Spikelet

The number of spikelet per panicle varied significantly with genotypes under saline and non-saline conditions. The average number of spikelet produced in O. glaberrima was higher than in O. sativa (Fig. 2). However, O. glaberrima genotypes showed 24.1% reduction in percentage of spikelet produced in salinized conditions. Four (4) O. sativa genotypes (SIPI 692033, ARG 6605, FARO 19 and Bouake 189) showed no reduction in spikelet per panicle. BG 2765, IR 77674-B-20-1-2-1-3-6-B, ITA 306 and IET 3137 however, revealed increase in spikelet number ranging from 9% in TOX 4004-3-1-2-1 to 46% in SAHEL 108, with an average general reduction of 11.43%.

3.12 Discussion

Rice genotypes showed varying degrees of tolerance to salinity stress within and between species. Some degree of cultivar tolerance to salinity and other abiotic stress is available in rice germplasm [21]. The rice plant is relatively susceptible to salinity at seedling stage, gains good tolerance at the tillering stage, but becomes very susceptible at the flowering stage [22]. Tolerant genotypes showed lower reductions in characterized traits than in susceptible genotypes. Reductions in all observed plant biometics due to salt stress could largely be attributed to the fact that under salt stress, NaCL uptake hinders the uptake of water and minerals. The ability of these plants to sequester salts in the leaves and roots therefore obstructs normal metabolism and hence affects plants physiology and growth mechanisms [9]. Results obtained suggests that the rapid shoot elongation, improved tillering and increase in plant biometrics of genotypes observed under saline conditions might have been due to competition for energy required for maintenance processes for survival and hence increasing its photosynthesizing ability. A reduction on days to maturity under salt stress further corroborates increasing energy utilization in order to escape salinity. Therefore, under salt stress, the physiological mechanisms were more or less devoted to yield and yield component parameter in other to escape the stress factor. These findings were corroborated by several researches on salinity tolerance where it was implied that some genotypes exhibited reductions to days to maturity, plant height and increased number of tillers [23] under salinized conditions [24]. Significant increase in tiller number under saline conditions as compared to non-saline condition at reproductive stage have been opinionated as an act of escaping stress [25].

Incidence of lodging, days to maturity, panicle length and culm number were most pronounced in O. glaberrima genotypes while reductions in plant height, culm length, leaf length and width were highly exhibited in O sativa genotypes. These differences in response to salt stress could be attributed to varying effect of salinity on the genotypes, variations among genotypes and variations between species. The increase in grain length and brown rice length in few tolerant genotypes that failed to show commensurate reduction in grain width and brown rice width changed the grain shape of these genotypes. This suggests that salinity stress results in a greater reduction in grain length than width and that greater reduction occurs in wild genotypes than cultivated species. These reductions and changes in grain shape also greatly affect grain yield of genotypes at reproductive stage. This is in concordance with results that stress affects grain processes since rice was highly sensitive to salt stress during reproduction [26]. However, greater reduction in grain length and width in O. glaberrima could result from the fact that salinity affects this species more than O. sativa which have been cultivated and domesticated in varying ecotypes and thus less responsive to the stress factor.

Considerable effects due to salinity were observed for the yield parameters like filled grain, spikelet per panicle, spikelet number, grain length and width, brown rice length and width and brown rice shape. This is because salinity affected the yield and yield component at reproductive stage rather than at the vegetative or seedling stage because of the bid to attain early maturity thereby compromising it critical developmental periods. These yield components have been reported to have their own critical development periods that can affect final grain yield [27]. Similarly, it was further reported that yield reduction caused by osmotic stress was mainly attributed to the decrease in percentage of filled spikelet and the number of filled grains per panicle [25,28]. The yield attributes such as reproductive tillers, panicle length, number of spikelets per plants, number of filled grains per panicle, 100-grain weight were found to be higher in tolerance than susceptible genotypes. The reduction in yield of the genotypes under salt stress might be due to decrease in enzyme activity and reduced photosynthetic activity [29]. It was also opinionated that yield reduction was due to the decrease in the number of filled grains per panicle [30]. Similar effects of salt stress on yield attributes have been reported [31]. Further support that the high yielding varieties performed best and had a higher grain yield than

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susceptible genotypes due to higher number of panicles, number of filled grains per panicle and 100-grain weight [32] have been reported. The reductions in these characters were also significantly higher in O. glaberrima than in O. sativa. The increase in panicle length exhibited by O. glaberrima species did not culminate to higher grain yield under salt stress due to an overwhelming number of aborted spikelets per panicle, unfilled grain and reduced number of filled grains. These reasons were also in accordance with confirmatory results that higher number of panicles, filled grains per panicle and grain weight positively associated with increased yield [33,34].

The higher significant correlation between yield attributes, plant height and SES score may indicate that these characters plays significant role in the salt tolerance of genotypes. The negative association between brown rice shape and grain weight indicate that bold to medium shape genotypes resulting from increased grain length also fundamentally contributes to yield. Similarly, positive associations between the numbers of spikelet's, unfilled grain, grain length and leaf length also shows that increased leaf length (flag leaf) of genotypes also plays important role in determining tolerance and susceptibility of genotypes to salt stress. Thus the reduction of leaf length could be a major cause of reduced number of spikelet and unfilled grains thus resulting in reduced grain yield. Characters such as brown rice shape, grain length and brown rice length exhibited negative but significant correlation with grain yield. Positive and significant correlations among reduction in number of filled grains and grain yield at reproductive stage in 80 RILS of POKKALI and IR 29 [22] which was not in conformity of results obtained in this study, as increase in number of filled grain was tantamount to higher yield .

4. CONCLUSION

The effect of salinity stress on plant growth and yield were genotype and specie dependent. Salinity adversely resulted in reductions in plant biometrics. However, degrees of growth plasticity were observed in some genotypes as an escape strategy against salinity. Salt stress induced changes in grain lengths and seed shape. The presence of flag leaf and penultimate leaf or few leaves before panicle initiation determined the genotype ability to set seed at reproductive growth stage.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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