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Evaluation of Salt Tolerance in Ten different Rice Genotypes at Seedling Stage

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ABSTRACT: Initially 10 rice genotypes were evaluated for their tolerance response to salt tolerance at seedling stage in *rabi* 2019 in the net house of ICAR-NRRI, Cuttack. Amongst all FL478 was found to have least reduction in plant vigour and biomass followed by AC41585 and AC39416A. While some genotypes like Rashpanjor, CSR27 and Binadhan 8 were considered as moderately tolerant with a SES score of 5 and moderate retention of leaf water potential and plant dry matter. But susceptible genotypes like IR29 (check), Sabita and Sadri were observed to have highest reduction in vigour and biomass. Higher accumulation of noxious amount of Na^+ inside the leaf mesophyll cell hampered the chlorophyll pigment concentration in the susceptible genotypes. But maintaining lower Na^+ and high retention of K^+ helps some tolerant genotypes (FL478, AC41585 and AC39416A) to survive the salt stress in seedling stage. From this study, we conclude that an efficient management of noxious Na^+ ion in the photosynthetic tissue helps to render greater salt tolerance in early growth stages.

Keywords: Salt stress, SES Score, Na^+, and K^+ concentration, chlorophyll concentration.

INTRODUCTION

Many abiotic stresses have negatively affected the grain production and threaten the food security worldwide. Amongst them soil salinity is one of the major reasons that affects about 50% of the irrigated land (Fita *et al.*, 2015). Rise in mean sae level, faulty irrigation and poor drainage lead to salt intrusion in agricultural land. Around one third of irrigated land and about 20% was affected by salinity (Munns, 2005) and out of that approximately 6.73 million hectares of irrigated land are affected by salinity in India alone (Singh *et al.*, 2008). Rice is one of the most important cultivated food grain, feeds more than half of the world's population and provides about 50-80% of daily calories requirement. But unfortunately several complications including climatic concerns limit rice productivity (Jyothi *et al.*, 2022). Like many glycophytes, rice can tolerate salinity up to 3-4 dS m^{-1} above which around 10-30% of severe yield loss was recorded. In whole life cycle it is relatively tolerant during germination and active tillering but very sensitive at early vegetative and reproductive stage to salinity (Ahmadizadeh *et al.*, 2016). Plants experience both "osmotic and ionic stress" due to reduction in water potential of the soil solution and subsequent hyper accumulation of toxic Na^+ in the cells adversely affects the cellular ionic equilibrium (Munns and Tester 2008). This severely disturbs the essential metabolic and physiological processes and leads to severe loss of cholorophyll content, leaf area, plant biomass and other growth factors (Ismail *et al.*, 2007; Baker 2008). Salt stress reduces the ability of plants to take up water and this quickly causes reduction in growth rate (Soujanya *et al.,* 2022). To encounter with the ill effects of salinity plants employed different adaptive strategies to survive the adversity and maintain a desirable level of water potential in the cell. Along with this to nullify the adverse impact of salinity and excess amount of Na^+ from xylem, plant system have adopted different strategies, which include Na^+ exclusion, K^+ retention and excess Na^+ sequestration (vacuolar sequestration) (Munns and Tester 2008). Keeping this in mind, our study is completely focused to identify the novel genotype tolerant to salt stress at early seedling stage with minimum Na^+/K^+ ratio, maximum pigment integrity and vigour retention.

MATERIAL AND METHODS

A panel of ten genotypes was taken including one susceptible (IR29) and one tolerant (FL478) checks for this investigation. All ten genotypes (FL478, AC41585, AC39416A, Sadri, Rashpanjor, CSR27, Binadhan8, Luna Suvarna, Sabita and IR29) were grown in the net house of the Crop Physiology and Biochemistry division of ICAR-NRRI, Cuttack (85°552′48″E–

85°562′48″E and 20°262′35″N–20°272′20″N) in *rabi* 2019. The assessment of salt tolerance was done in hydroponics by Factorial CRD experimental design, where one factor was treatment (control and stress) and another one was genotype. The seeds were surface sterilised with 70% ethanol and repeatedly washed with distilled water and placed on moistened paper within petri plates for 2 days in the dark for better germination. The germinated seeds were then planted into the floating 10 ×10 styrofoam panel, one seed in each hole. The styrofoams were placed on the trays filled with Yosidha nutrient solution (Gregorio *et al*., 1997). The pH of 5.0 being periodically maintained till the plants attained a desirable vigour. After two weeks one set of plants was subjected to salt stress of 12 dS m^{-1} and another set of plants was grown normally in the Yosidha solution. The plants were kept under salt stress until most of the IR29 genotype (Susceptible check) got a Visual Salt Injury score of 9 (SES score, IRRI).

Visual Salt Injury (VSI) score. All the 10 genotypes were given an SES score by following the standard protocol developed by IRRI (Gregorio *et al*., 1997) in response to salt stress. A plant with no visible signs of damage was given a score of 1 and was considered to be extremely tolerant, while a very susceptible plant with significant damage to the stalk and total chlorosis of the leaves was given a score of 9. The tolerant, moderately tolerant, and susceptible genotypes were given the intermediate SES scores of 3, 5, and 7 respectively. The SES scoring was done for all the hydroponics trays under both controlled and stressed conditions individually.

Total plant biomass (g), root and shoot length (cm). For evaluation of total plant biomass and plant vigour at seedling stage, three plants from each of the genotypes were collected from the hydroponics solution after IR29 attained an SES score of 9. Samples in three replicates were kept in the oven at 80°C for 7-8 days until the samples were completely dried. After complete drying, the weight of the individual plant was taken with the help of a weighing machine and the mean was expressed in terms of mg. Both root and shoot length was measured at for each genotype × treatment combination. The shoot and root were separated manually and the length was measured with the help of a scale and expressed in centimetres (cm).

Leaf water potential (Mpa). Leaves from each genotype × treatment combinations were collected for measuring the leaf water potential. The leaves were cut into small discs (0.5 cm diameter) using scissors. The small leaf discs were placed on the disc chamber of the psychrometric water potential system (PS9PRO water potential system, Wescor, United States). The data was taken from the screen of the system after waiting for about 5 minutes. The water potential was expressed in terms of mega pascals (Mpa)

Total chlorophyll content. The total chlorophyll content was estimated by the method given by Arnon (1949) from each genotype × treatment combination after the imposition of stress. The leaf samples were collected from the 2nd leaf from the top. Fresh leaves of about 25 mg were collected and cut into small pieces. The pieces were taken in test tubes of 10 mL 80% v/v acetone and incubated for 48 hours in dark. After 2 days the absorbance of the extract was taken at 645 and 663 nm in a UV spectrophotometer (UV 2600, Shimadzu, Japan).

Estimation of tissue Sodium (Na^+), Potassium (K^+) concentration. Assessment of tissue Na^+ and K^+ concentration of whole seedlings was done. The plants were taken out of the hydroponics solution after the SES score of IR29 reached the value 9. The fresh leaves (2nd leaf from the top), stem and roots were separated carefully and dried in an oven at 60°C for a week. For the estimation of tissue ion content (both Na^+ and K^+), extraction of 50 mg of the dried sample of each type from three replications was powdered and macerated in 50 mL of 0.1N HCl and kept for 48 hours. The extracts were then filtered by using Whatman #40 filter paper. The Na^+ and K^+ content in the extract measured by using a Flame Photometer (JENWAY PFP7 Photometer of Cole-Parmer scientific experts, India).

Statistical analysis. The experiment was conducted in a completely randomized design, and the data were subjected to one-way factorial ANOVA. A post hoc analysis for pair-wise comparison of treatment × genotype combinations were performed by Tukey's multiple comparison tests.

RESULT AND DISCUSSION

The results of the present study showed that, salinity has a very detrimental effect on rice plants' health at the early seedling stage. Based on visual salt injury the plants were assigned with an appropriate SES score of 1 to 9. FL478, AC41585, and AC39416A having an SES score of '3' were considered to be tolerant to salt stress. While CSR27, Rashpanjor, Binadhan 8, and Luna Suvarna were considered moderately tolerant with a score of '5'. While two genotypes, Sadri and Sabita were seen to have stunted growth along with some severe symptoms of salt stress and were considered moderately susceptible with a score of '7'. Whereas almost all plants of IR29 were dead and found to be highly susceptible with a score of '9'. The productivity varies considerably with the reduction in biomass and vigour (Ali *et al*., 2014). In reciprocation to score significant reduction in both shoot and root length was observed under salt stress was observed. Shoot length was highly decreased in susceptible genotype IR29 (28.49%) as compared to its control. In moderately tolerant genotypes like Rashpanjor, CSR27 and Luna Suvarna about 20% reduction in shoot length was observed. While least reduction was recorded in FL478 (8.15%) followed by AC41585 (12.92%) and AC39416A (15.45%). Similarly, more than 30% reduction was observed in the root length of IR29 and Sabita, whereas, a little less, yet significant reduction was observed in Rashpanjor (18.65%) and CSR27 (22.90%), Luna Suvarna (21.24%) and Binadhan 8 (24.62%). The lowest reduction was observed in FL478 (8.48%) followed by AC41585 (12.63%) and AC39416A (15.91%). Similarly significant reduction in total biomass observed in all the genotypes after 7 days of imposition of salt stress. The least reduction of biomass was observed in FL478 (17.67%) followed by AC41585 (22.22%) and AC39416A (26.47%). The highest decline of more than 50% in biomass was recorded in susceptible genotype IR29. Hence genotype with a greater ability to retain vigour and biomass under stress showed better salt-tolerance ability (Singh and Flowe 2010). Highest retention in LWP was recorded in tolerant genotypes AC41585 (-2.45 MPa) was at par with FL478 (-2.49 MPa) and Rashpanjor (-2.25 MPa) and followed by AC39416A (-2.66 MPa). Less but significant decrement in LWP was observed in moderately tolerant genotypes like CSR27 (-3.14 MPa), Binadhan 8 (3.57 MPa) and Luna Suvarna (-3.49 MPa). Maximum drop in water potential was recorded in IR29 (-5.66 MPa) followed by Sabita (-4.79 MPa) (Hossain *et al.,* 2015; Nounjan 2018).

Salinity severely disintegrates the structure and function of chlorophyll. The reduction in total chlorophyll content under salinity was reported in several plants. In mustard (Mittal *et al*., 2012), rice Sarkar *et al.,* 2013), and sugarcane (Cha-um *et al*., 2012) the reduction in the chlorophyll content was due to the deposition of a noxious amount of Na^+ in the leaf mesophyll tissues. Significant reduction in chlorophyll concentration was observed in all the genotypes as compared to control under salt stress. Irrespective of genotypic variation in chlorophyll concentration highest reduction was observed in IR29 (55.32%) followed by Sabita (49.73%) in the early seedling stage. In moderately tolerant genotypes like CSR27, Binadhan 8 and Luna Suvarna ~20-30% reduction in chlorophyll concentration was observed. While in FL478 (12.45%) the decrement was least among all the genotypes and followed by AC39416A (15.15%), AC41585 (18.13%) and Rashpanjor (20.18%). Many studies have reported that the maximum decrement in leaf water potential and relative water content was drastically decreased in sensitive genotypes Increment in tissue Na^+ concentration was significant in the root, stem and leaves of all the genotypes was observed. Rapid Na^+ buildup causes ion toxicity and nutrient imbalance and reduces plant growth and development after 14 days of salt stress (Mousa *et al*., 2013). In our study the highest root Na^+ concentration was observed in FL478 (319.10 mg kg^{-1}, DW), which was at par with AC41585 (304.99 mg kg^{-1}, DW) and AC39416A (307.19 mg kg^{-1}, DW). In genotypes like Sabita (191.57 mg kg^{-1}, DW) and IR29 (217.99 mg kg^{-1}, DW) the root Na^+ concentration was comparatively less than in other genotypes. In stem portions also significant increment in Na^+ concentration was noticed in all the genotypes as compared to control plants under stress. However, the lowest Na^+ accumulation was noticed in the stem region of AC41585 (211.58 mg kg^{-1}, DW) which was at par with AC39416A (214.02 mg kg^{-1}, DW), CSR27 (219.98 mg kg^{-1}, DW) and Binadhan 8 (216.40 mg kg^{-1}, DW). The highest stem Na^+ concentration was observed in Rashpanjor (274.89 mg kg^{-1}, DW). But in the leaf tissues a different pattern of Na^+ accumulation was observed as compared to root tissues. Least Na^+ was accumulated in the photosynthtically active tissues of FL478 (130.89 mg kg^{-1}, DW) followed by AC41585 (141.22 mg kg^{-1}, DW) and AC39416A (145.07 mg kg^{-1}, DW). A moderate amount of Na^+ was deposited in the leaves of CSR27 (172.81 mg kg^{-1}, DW), which was at par with Binadhan 8 (180.33 mg kg^{-1}, DW) and Luna Suvarna (181.76 mg kg^{-1}, DW). However, maximum Na^+ concentration was found in the leaf tissues of IR29 (343.09 mg kg^{-1}, DW) followed by Sabita (306.99 mg kg^{-1}, DW) under 12 dS m^{-1} salt stress. Findings of some studies revealed the existence of strong correlation between salt exclusion and salinity tolerance (Munns *et al*., 2006). This is what exactly supports the fact that salt-tolerant genotype like FL478 and AC41585 excludes excess Na^+ through roots or restricts the upward movement of Na^+ to the leaves, The failure of this discrimination process ingenotypes like IR29 and Sabita perhaps led to its susceptibility under 12 dS m^{-1} salt stress at the seedling stage.

Similarly tissue K^+ concentration varied significantly in all the genotypes under salinity. A drastic reduction in K^+ concentration in root, stem, and leaf tissues were observed in stressed plants as compared to the control. Least K^+ concentration was observed in root tissues of susceptible genotypes like IR29 of 154.06 mg kg^{-1}, DW and followed by Sabita (155.09 mg kg^{-1}, DW). Whereas maximum K^+ retention was observed in the roots of FL478 (180.59 mg kg^{-1}, DW) and was at par with AC41585 (178.37 mg kg^{-1}, DW), AC39416A (168.50 mg kg^{-1}, DW) and Rashpanjor (162.14 mg kg^{-1}, DW). It was seen that the presence of better selectivity and retention capacity for K^+ under a high load of Na^+ is another crucial factor of tolerance under salt stress (Munns and Tester 2008). A similar kind of trend was observed in both stem and leaf tissues. The highest leaf K^+ acquisition was observed in AC41585 (348.69 mg kg^{-1}, DW) followed by FL478 (341.09 mg kg^{-1}, DW), AC39416A (320.46 mg kg^{-1}, DW), and Rashpanjor (317.07 mg kg^{-1}, DW). We also found that the susceptible genotypes (IR29 (171.13 mg kg^{-1}, DW), Sabita (222.26 mg kg^{-1}, DW) were incapable of maintaining a proper ionic balance under prolonged salt stress which leads to susceptibility (Reddy *et al*., 2017).

Table 1: Effect of salt stress on SES score, Plant dry weight (g), shoot and root length (cm) and leaf water potential (mPa).

Genotype	SES Score	Plant DW(g)		Shoot length (cm)		Root length (cm)		LWP (mPa)	
	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress
FL478	3	0.297	0.245 (17.67%)	21.46	12.23 (8.15%)	8.41	7.70 (8.48%)	-1.69	-2.49
IR29	9	0.209	0.095 (54.50%)	21.74	15.37 (28.49%)	8.34	5.60 (32.88%)	-1.49	-5.66
AC41585	3	0.341	0.265 (22.22%)	29.50	16.58 (12.92%)	10.61	9.27 (12.63%)	-1.69	-2.45
Sadri	7	0.309	0.180 (49.79%)	27.81	17.74 (20.52%)	10.64	7.40 (30.47%)	-1.65	-4.49
AC39416A	3	0.376	0.276 (26.47%)	29.89	16.78 (15.45%)	11.69	9.83 (15.91%)	-1.50	-2.66
Rashpanjor	5	0.284	0.195 (31.31%)	31.22	17.78 (19.65%)	11.91	9.70 (18.56%)	-1.39	-2.25
CSR27	5	0.345	0.235 (31.91%)	22.75	13.87 (20.04%)	8.65	6.67 (22.90%)	-1.34	-3.14
Binadhan 8	5	0.247	0.168 (32.18%)	24.79	15.23 (22.32%)	8.62	6.50 (24.62%)	-1.57	-3.57
Luna Suvarna	5	0.323	0.208 (35.56%)	28.31	16.65 (21.27%)	9.23	7.27 (21.24%)	-1.20	-3.49
Sabita	7	0.352	0.207 (41.26%)	27.37	17.52 (26.22%)	10.76	7.37 (31.52%)	-1.66	-4.79

	SES Score	Plant DW(g)			Shoot length (cm)			Root length (cm)			LWP (mPa)		
	GxS	G	S	GxS	G	S	GxS	G	S	GxS	G	S	GxS
SE(M)±	0.516	0.003	0.001	0.004	0.660	0.295	0.934	0.324	0.145	0.459	0.59	1.40	0.81
LSD (p<0.05)	1.523	0.008	0.004	0.011	1.887	0.844	2.669	0.927	0.415	1.311	1.87	0.79	2.59

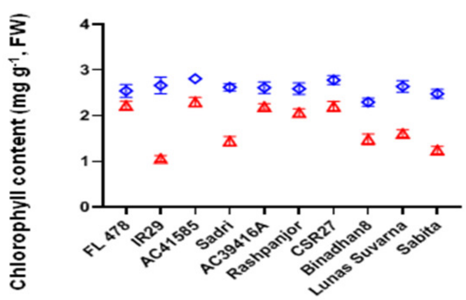

Fig. 1. Effect of salt stress on chlorophyll concentration (mg g^{-1} FW) in rice.

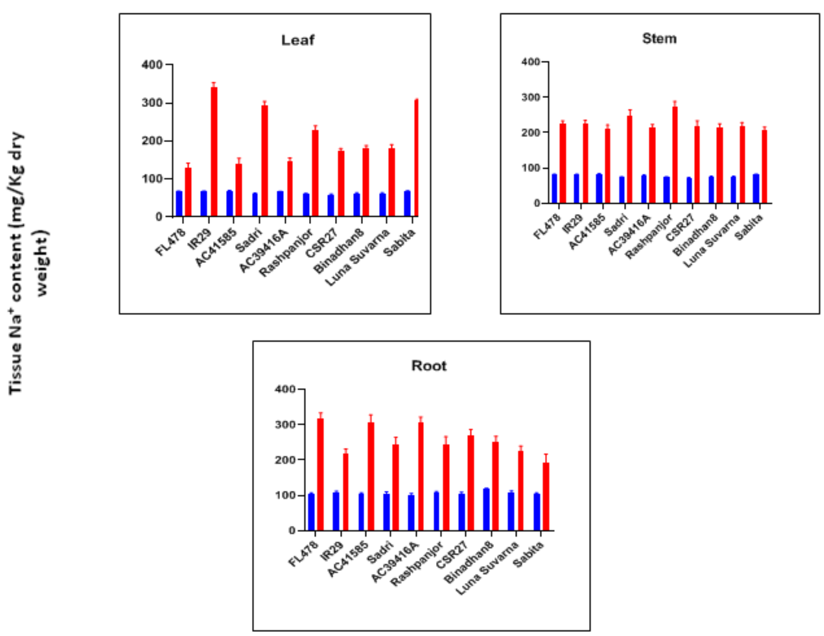

Fig. 2. Effect of salt stress on Na^+ concentration (mg kg^{-1} DW) in rice.

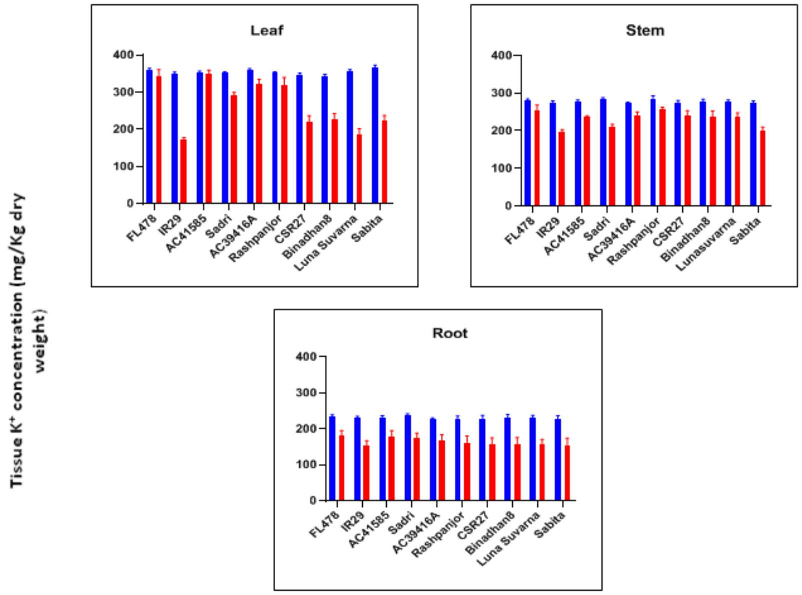

Fig. 3. Effect of salt stress on K^+ concentration (mg kg^{-1} DW) in rice.

CONCLUSIONS

Hence it can be concluded that, genotypes (FL478, AC41585 and AC39416A) with least Na^+ accumulation and maximum retention of K^+ were able to retain integrity of photosynthetic pigments (chlorophyll) and able to maintain a stability in ionic homeostasis inside the actively growing plant tissues even under salinity. On the other hand susceptible genotypes (IR29 and Sabita) failed to do so hamper the overall plant vigour and bio mass retention.

FUTURE SCOPE

Genotypes with greater selectivity towards K^+ than Na^+ could possibly retain more biomass, water potential and pigment stability, which is one of the key trait adapted for screening salt tolerance at seedling stage.

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Conflict of Interest. None.

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