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Full Length Article

Adaptive Behaviour of Roots under Salt Stress Correlates with Morpho-physiological Changes and Salinity Tolerance in Rice

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Abstract

Salinity screening of five commercially available rice (*Oryza sativa* L.) genotypes *i.e.*, KS-282, Super Basmati, KSK-133, Shaheen Basmati and DilRosh was performed at 100 m*M* sodium chloride (NaCl) concentration to investigate the effect of salt stress on root architecture and to understand the role of roots in salinity adaptation. Morpho-physiological attributes and water conservation level were monitored in all genotypes under control and stress conditions. Among the tested genotypes, KS-282 was found to be fairly tolerant while Super Basmati was found to be highly sensitive. Changes in the root architectural dynamics were analyzed using WinRhizo pro software program. Na^+ and K^+ content in the roots and shoots were correlated with morpho physiological growth parameters and variations in root geometry. A significant decrease in the root length, lateral root density combined with reduced surface area was evident in the tolerant genotype within the first three days of salt treatment. Growth was sustained gradually after this initial delay while no such response was recorded in the sensitive genotype. Similarly, Na^+ content was found to be significantly lower in the roots and shoots of the tolerant genotype as compared to the sensitive plants. Our data highlight a primal reduction in the total root length and surface area as a major component in tolerance against Na^+ accumulation and salinity induced damage. Our data can be used to understand root growth and adaptation in other rice species as well as many other forage grass and cereal species under salt stress. © 2019 Friends Science Publishers

Keywords: Hydroponics; Salt stress; *Oryza sativa*; Root system; WinRhizo pro

Introduction

In plants, roots serve a central role in the absorption of water, dissolved ions and nutrients (Kano *et al.*, 2011). It is also the first organ that encounters soil born biotic and abiotic stress. In soil environment, changes in the root physiology and direct observation is a challenging task as sample acquisition procedure may damage fine root structure (Wang *et al.*, 2015). Root system and its architecture is largely under genetic control but can be significantly influenced by environmental factors (Fitter, 2002). Understanding changes in the root depth and branching in response to salt stress maybe very helpful in describing the role of roots not only in ion uptake as well as control of water loss through areal parts of the plant. To describe the root morphology, several parameters can be taken into account such as total fresh/dry weight, length and number of primary and secondary roots or the total surface area. Microscopic observations may also be an important tool in describing the root anatomy; however, these parameters are not sufficient to explain the detailed root system especially in monocotyledonous plants due to a fine and fibrous roots. These roots are highly flexible and they have the potential to change their growth rate and morphology in response to changes in the osmotic and ionic content of soil (Wang *et al.*, 2009).

Rice is a highly salt sensitive monocot feeding millions of people globally. Seedling and reproductive phase of growth are the most sensitive stages under saline conditions (Lutts *et al.*, 1995; Zeng and Shannon, 2000). Salt exerts its toxicity by inducing osmotic, ionic and oxidative stress (AbdElgawad *et al.*, 2016; Akram *et al.*, 2017) primarily at the level of roots ultimately compromising a number of morpho-physiological growth factors (Yeo *et al.*, 2018). Many physiological parameters along with ion distribution/compartmentalization patterns have been studied by various groups in rice to understand salinity tolerance (Formentin *et al.*, 2018; Mumtaz *et al.*, 2018) but little is known about the role of root architecture and exact mechanisms of water and ion uptake under salt stress. Previous studies on rice root morphology (Li, 1979; Kawada, 1984; Gu *et al.*, 2017) under different management conditions provide insufficient information about the root dynamics and its role in improving salinity adaptation.

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For the present study we hypothesize that an early response of the tolerant genotype at the level of roots namely through a marked reduction in total root length, surface area, average diameter and number of tips can explain the differences in toxic ion uptake and distribution pattern in the roots and shoots. For test this hypothesis, we tested five commercially important indica rice genotypes *i.e.*, KS-282, Super Basmati, KSK-133, Shaheen Basmati and DilRosh (Basmati and Non-basmati) against 100 m*M* NaCl stress.

Materials and Methods

Plant Material and Growth Conditions

Mature seeds of five commercially available Indica rice (*Oryza sativa*) genotypes were obtained from the Rice Breeding Program, National Agriculture Research Center (NARC) Islamabad, Pakistan. Seeds were surface sterilized and inoculated on moist filter paper in plastic petri plates and then placed in dark at 26±1ºC temperature with 70% humidity for germination. Seeds with 2 mm length radical were selected. Hoagland nutrient solution was added to hydroponic containers. Seeds were sown in ager in seed holders of hydroponic cover lids and placed in growth chamber at 26±1ºC temperature with 70% humidity and 18 h photoperiod. Water level and pH (5.8) was maintained over the experimental period.

Salinity Treatment

After seven days in hydroponic tanks, plants produced fully expended 2nd leaf. Plants with similar height and appearance were selected for treatment application. The treatment group was exposed to 100 m*M* NaCl. No salt was added to the nutrition solution of control group.

Changes in the Morpho-physiological Attributes under NaCl Stress

Growth parameters including length of intercolar, emergence and average length of 2nd, 3rd and 4th leaf were monitored after every 24 h starting from the day 1 of salt treatment up to day 7. Five plants were selected randomly from each hydroponic container to measure the fresh and dry weight of shoots and roots at the end of experiment. Plant mortality rate was measured by counting the number of dead plants on day 7. Three replications were used for each entry.

Water Conservation and Hydraulic Conductivity

Stomatal assay: Fully grown 2nd leave (in triplicate) were used from each genotype. The stomata were observed under Leica, DM 500 stereo microscope at two time points (24 and 48 h) from control and 100 m*M* NaCl treated tanks. Images of stomata were taken and analyzed using ImageJ software (https://imagej.nih.gov/ij/). Images of 25 stomata per leaf were recorded. Experiment was performed twice and data was analyzed using Student's t-test.

Measurement of relative water content: Second leaf (5 cm^2) sections were cut from the control and treatment group plants at 24 and 48 h. After taking their fresh weight, immediately they were fully immersed in water for full hydration for at least 4 h at 26±1ºC. After attaining full turgidity, the leave sections were surface dried with tissue pare and weighted to obtain fully turgid weight (TW). The leaves were then oven dried at 40°C for 3 days to obtain the dry weight (DW). Relative water content was calculated using the formula (Barr and Weatherley, 1962):

$$RWC (\%) = [(W-DW)/(TW-DW)] \times 100 \ (n = 6)$$

The experiment was repeated thrice with five leaves per plant per control and treatment group.

Selection of Contrasting Genotypes against 100 m*M* NaCl Stress for Analysis of Root Architecture and Ion Distribution Pattern

Based on the highly contrasting morpho-physiological data and plant mortality rate at 100 m*M* NaCl stress, KS-282 and Super Basmati genotypes were selected for analysis of changes in the root geometrical dynamics and Na^+/K^+ ion distribution pattern in the roots and the shoots. Plants were grown hydroponically to avoid any damage to roots during sampling. Impact of salt stress on root growth rate and morphology was correlated with the variation in water loss, morpho-physiological performance and Na^+/K^+ ion distribution pattern in the roots and shoots.

Digital Scanning of Roots

Changes in the average length of nodal and lateral roots, their surface area, total diameter and number of tips were monitored every 24 h from day 1 of salt treatment till 7 days by obtaining Grayscale images (600 DPI) of roots using a high definition optical scanner. Image analysis was performed using WINRHIZO Pro (Regent Instruments, QC, Canada) software. Experiment was performed with five roots per plant. Average total length, surface area, diameter and number of tips were obtained by random sampling and statistical comparison method.

Na^+ and K^+ Distribution

Plants were harvested at day 1 and day 3 to collect roots and shoots. The sample were oven dried for 4 days at 40°C and ground to a fine powder which was dissolved in 68% HNO_3 (Sigma Aldrich, Germany). The samples were diluted and analyzed using Atomic absorption spectrophotometer (Perkin Elmer Model 303). Na^+ and K^+ concentration was determined (mg/g of dry weight) in roots and shoots by comparing to the known concentrations of cation standards.

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Statistical Analyses

All experiments were repeated three times with n=54. Student's t test and ANOVA followed by Post-Hoc Fisher's Least Significant Difference (LSD) test were applied to experimental data to determine the differences between control and treatment groups.

Results

Influence of Salt on Intercollar Length, Leaf Length and Plant Mortality Rate

Growth rate of all five genotypes was affected by salt stress to varying degree. Amongst the five genotypes, KS-282 was least affected being most tolerant while Super Basmati was found to be the most sensitive genotype with a significantly marked reduction in the 1st intercollar length (Table 1), emergence and average length of 3rd and 4th leaf and plant mortality rate (78%) at day 7 of salt treatment (Table 2). KSK-133 and DilRosh were moderately tolerant (mortality rate 42% and 38% respectively) while Shaheen Basmati was moderately sensitive (mortality rate 62%). The fresh and dry weight of shoots and roots of all genotypes were affected under salt stress (Table 3) with least effect on KS-282 and significantly greatest reduction in the fresh and dry weight of shoots and roots of Super Basmati.

Influence of Salt on Stomatal Conductance and Relative Water Content of 2nd leaf

A significant increase in the length of stomatal aperture was observed in KS-282 and KSK-133 at 24 h of salt treatment (Fig. 1a). An increase in the length indicates more closed stomata while shorter size presents an open conformation. On the other hand, no change in the size of stomatal aperture was recorded in case of Super Basmati, Shaheen Basmati and DilRosh (Fig. 1a). A more closed conformation of stomata with a significant increase in the length was observed in KS-282, followed by KSK-133 at 48 h of treatment. Similarly, Shaheen Basmati and DilRosh were also able to respond at this time point with more closed stomatal aperture (Fig. 1b). No change in the size of stomatal aperture of Super Basmati was seen indicating its failure to respond to stress at both time points to stop water loss through transpiration or a control over stomatal guard cells under salt stress (Fig. 1a, b and Fig. 2). This observation was further supported by data obtained in our study on relative water content with KS-282 having the least and non-significant decrease in relative water content of the second leaf at 48 h of salt treatment in comparison to the control plants, followed by KSK-133 and DilRosh (Fig. 3b). No change in the relative water content of all genotypes was observed at 24 h of salt treatment (Fig. 3a). Maintenance of the water content under stress forecasts a control over transpiration during salt stress to maintain turgor pressure.

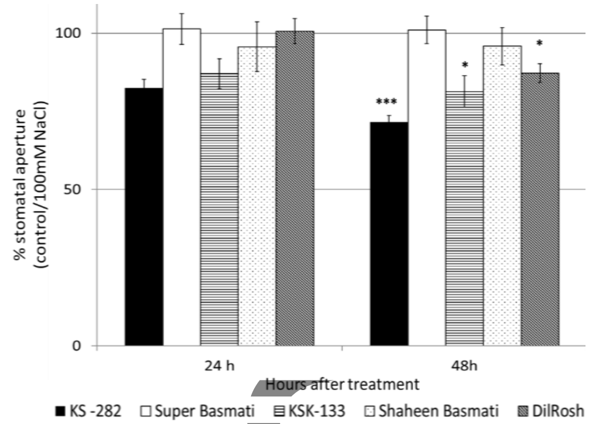

Fig. 1: Influence of salt stress on stomatal aperture of fully expanded 2nd leaf of selected rice genotypes. Change in the length of stomatal aperture of second leaf of five rice genotypes at 24 h (a) and 48 h (b) after treatment with 100 m*M* NaCl. Values represent the mean ± SE of three independent experiments in duplicate. Different letters indicate statistically different expression levels. Means followed by the same letter are not different at $p<0.05$ by Fisher's LSD

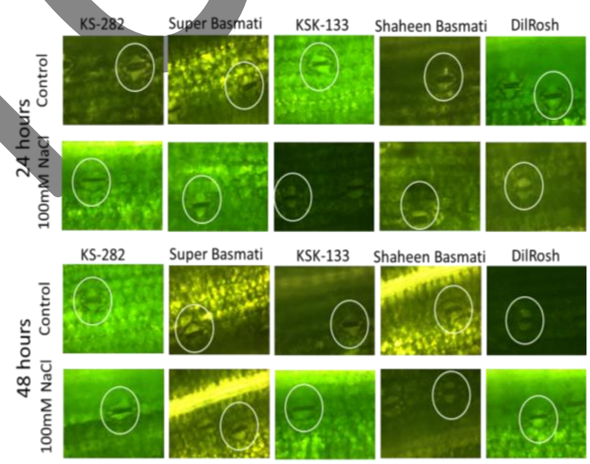

Fig. 2: Influence of salt stress on length of stomatal aperture of fully expanded 2nd leaf of all genotypes. Analysis of stomatal aperture revealed more closed stomata (large stomatal aperture length) of the 2nd leaf in KS-282 at 24 h of salt treatment. 48 h after salt treatment a significant increase in the size of stomatal aperture (more closed stomata) was evident in KS-282, KSK-133 and DilRosh. No significant change in the size of stomata of Super Basmati and Shaheen Basmati was observed by microscopic observation and image analysis by using image J software

However, a highly significant decrease of relative water content was observed in second leaf of Super Basmati and Shaheen Basmati plants respectively (Fig. 3b) showing greater water loss and reduced turgidity.

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Table 1: Influence of salt stress on intercolar length of different rice genotypes

Treatment	1st intercolar length (3 days)		2nd intercolar length (3 days)		1st intercolar length (7 days)		2nd intercolar length (7 days)	
100 m*M* NaCl	-	+	-	+	-	+	-	+
KS-282	3.1±0.02	3.1±0.03^{ns}	0.2±0.02	0.3±0.1^{ns}	3.3±0.01	3.2±0.5^{ns}	1.9±0.3	0.5±0.1*
Super Basmati	2.9±0.05	1.0±0.01**	nd	n.d	3.2±0.02	1.2±0.1**	1.6±0.2	n.d
KSK-133	3.2±0.01	2.9±0.05^{ns}	0.3±0.01	0.2±0.01^{ns}	3.4±0.01	3.0±0.01^{ns}	2.1±0.1	0.3±0.05*
Shaheen Basmati	3.1±0.05	2.0±0.01*	n.d	n.d	3.1±0.02	0.2±0.01**	1.5±0.2	0.5±0.02*
DilRosh	2.8±0.05	2.2±0.02^{ns}	0.5±0.03	0.5±0.01^{ns}	3.3±0.05	2.5±0.02^{ns}	1.8±0.01	0.7±0.01*

Average length of first and second intercolar at day 3 and day 7 with (+) or without (-) 100 m*M* NaCl treatment. Mean ± standard deviation with n=54. ** = significant at p 0.01, * = significant at p 0.05, ns = non-significant. n.d. = not developed

Table 2: Effect of salt stress on growth of third and fourth leaf and plant mortality rate of different rice genotypes

Treatment	3rd leaf length (3 days)		3rd leaf length (7 days)		4th leaf length (7 days)		Plant mortality (7 days)
100 m*M* NaCl	-	+	-	+	-	+	+
KS-282	8.4±0.7	9.1±0.8^{ns}	15.5±1.0	13.5±0.7^{ns}	13.8±0.9	6.4±1.3*	29%
Super Basmati	7.9±1.0	3.2±0.4**	14.1±1.2	5.3±1.4**	4.8±1.0	n.d	78%
KSK-133	7.2±0.5	6.2±0.5^{ns}	13.5±1.0	9.5±0.7	10.8±0.9	5.2±1.5	42%
Shaheen Basmati	6.1±0.4	5.9±0.5^{ns}	16.1±1.4	7.3±0.4**	2.8±1.0	0.9±0.5**	62%
DilRosh	8.8±0.3	6.9±0.4^{ns}	12.1±1.3	10.3±1.5^{ns}	7.7±0.4	2.4±1.2**	38%

Average length of 3rd and 4th leaf at 7 days after treatment with (+) or without (-) 100 m*M* NaCl. Mean ± standard deviation with n=54, ** = significant at p 0.01, * = significant at p 0.05, ns = non-significant. n.d= not developed

Table 3: Influence of salt stress on fresh and dry weights of roots and shoots of rice genotypes at day7 after salt treatment

Treatment	Shoot fresh weight			Shoot dry weight			Root fresh weight			Root dry weight		
100 m*M* NaCl	-	+	%Decrease	-	+	%Decrease	-	+	%Decrease	-	+	%Decrease
KS-282	0.577±0.02	0.376±0.001^{ns}	-34.84	0.173±0.01	0.122±0.02^{ns}	-29.48	0.215±0.01	0.156±0.007^{ns}	-27.44	0.015±0.002	0.012±0.002^{ns}	-20.00
Super Basmati	0.492±0.01	0.102±0.002**	-79.27	0.129±0.01	0.021±0.02**	-83.72	0.412±0.01	0.091±0.003**	-77.91	0.025±0.003	0.009±0.001**	-64.00
KSK-133	0.851±0.05	0.293±0.01**	-65.57	0.157±0.06	0.069±0.001*	-56.05	0.334±0.009	0.152±0.02**	-54.49	0.018±0.004	0.01±0.001*	-44.44
Shaheen Basmati	0.682±0.01	0.201±0.01**	-70.53	0.142±0.02	0.05±0.003*	-64.79	0.401±0.012	0.15±0.012**	-62.59	0.03±0.001	0.018±0.001*	-40.00
DilRosh	1.277±0.02	0.501±0.01*	-60.77	0.198±0.002	0.091±0.001*	-54.04	0.629±0.002	0.305±0.003*	-51.51	0.036±0.001	0.019±0.001*	-47.22

Fresh and dry weight of shoots and roots of five genotypes of rice at 7 days after treatment with (+) or without (-) 100 m*M* NaCl. Mean ± standard deviation. ** = significant at p 0.01, * = significant at p 0.05, ns = non-significant. n.d= not developed

Adaptive Response of Roots

A rapid decrease in the growth rate of roots in terms of total length, surface area and mean diameter was observed in an early response to salt stress in the tolerant plants. Root and shoot growth resumed later on in the tolerant genotype while no such response was observed in the root system of sensitive genotype and the plants showed early senescence and death.

Changes in the total root length in response to salt stress: The changes in the total root length of the two rice genotypes at different time points were analyzed using the data obtained from WinRhizo pro software (Fig. 4). In the tolerant KS-282 plants the increase in the root length was slow at day 3 (Fig. 5a). Afterwards, an increase in the length was accelerated and reached to optimal value by the end of experiment on day 7. In case of the sensitive plants, Super Basmati, the total root length increased rapidly in the control and treated plants till day 3 of salt exposure (Fig. 5b). However, after this rapid increase, growth was declined as the plants entered early senescence and death phase (Fig. 5b).

Decrease in the mean surface area in response to salt stress: The mean surface area of both tolerant and sensitive genotypes was affected under salt stress. The tolerant plants however responded by decelerating the increase in surface area initially with a deferment till day 3 of salt treatment (Fig. 5c) and then sustained a continuous increase in branching till day 7. In case of sensitive Super Basmati plants, no difference in the branching system and surface area was observed till the first 3 days of salt treatment but afterwards a consistently low surface area remained till day 7 (Fig. 5d).

Effect of salt on the average diameter of roots: A significant reduction in the average diameter, *i.e.*, the diameter of nodal and lateral roots was observed in both plant genotypes at day 3 of salt treatment (Fig. 5e and f). A consistently low mean diameter of sensitive Super Basmati plants up to day 7 of salt treatment (Fig. 5f) shows a failure of root system to adapt to salt stress and root abatement. While in the case of tolerant KS-282 plants, reduced average diameter along with markedly reduce total length and surface area presents a strategy to conserve resources and enhance adaption and survival.

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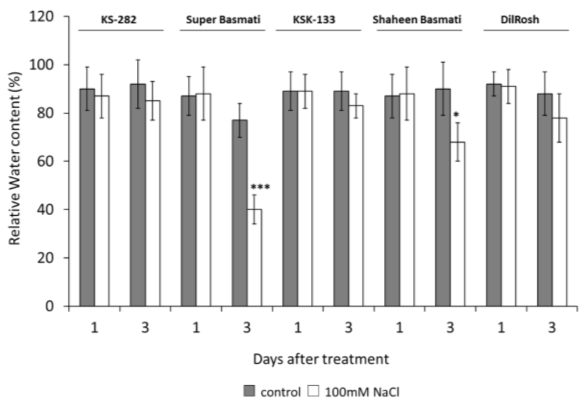

Fig. 3: Influence of salt stress on relative water content of second fully expanded leaf of different rice genotypes. Change in the relative water content of 2nd leaf (relative to the control) at day 1 (a) and day 3 (b) after treatment. Different letters indicate statistically different expression levels. Means followed by the same letter are not different at $p<0.05$ by Fisher's LSD

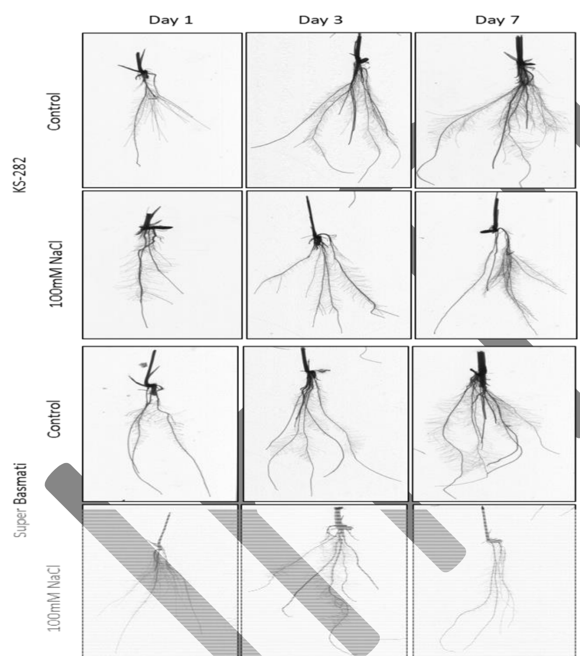

Fig. 4: Influence of salt stress on rice root architectural dynamics

Salt stress influenced all the selected parameters of both KS-282 and Super Basmati as revealed by digital scanning and analysis of roots by WinRhiro Pro software. KS-282 plants were able to reduce root develeopment within 3 days of salt tretment. After that initial delay plants recovered from stress and initiated a gradual root develeopmnet. Super Basmati plants, however were not able to respond to salt stress at root level and started early sensence after day 3 of salt stress

Changes in the total number of tips in response to salt stress: Number of tips in the root system of tolerant KS-282 reduced significantly initially but the plants entered a recovery state after day 3 (Fig. 5g). The number of tips was significantly lower than the control plants but increased continuously as the plants adapted to salt stress. While in sensitive plants, no significant change in the average number of tips was seen till day 3 of salt treatment. A substantial decrease was recorded however at the end of experiment on day 7 (Fig. 5h).

Na^+ and K^+ Distribution Pattern in the Roots and Shoot of Plant

The distribution and concentration of Na^+ and K^+ as measured in KS-282 and Super Basmati on day 1 and day 3 of salt treatment showed an increased uptake and flow of Na^+ from the roots to the areal parts of sensitive Super Basmati plants. Similarly, a low K^+ concentration was observed in the roots and shoots of sensitive plants after salt treatment (Fig. 6c and d) While in the tolerant genotype, KS-282, the flow of Na^+ from the roots to the shoots was restricted (Fig. 6b) to maintain a low Na^+ concentration in the shoots. Similarly, a higher K^+ concentration in the roots and shoots was observed (Fig. 6c and d) in the tolerant genotype (at day 1 and 3) as compared to the sensitive plants predicting a higher K^+/Na^+ ratio in plant body and hence better adaptation and survival under salt stress.

Discussion

Root is the main organs that explore water and nutritional matters in soil. Ionic contents and water status of soil impacts root architecture to ensure survival and growth (Julkowska *et al*., 2014). Changes in the root system under salt stress are an unexplored avenue and a largely ignored breeding objective. Lack of data on root dynamics in saline conditions gives room to investigate phenotypic changes in root development and plant survival under stress. Dynamics in root system may be correlated with the adaptive functional mechanism in the areal parts of the plants which are essential for a continued existence.

In our study, morpho-physiological assessment of rice genotypes in hydroponic tanks showed a decrease in growth determinants of all genotypes to a varying degree under the same salt concentration which matches with the findings of Rao *et al.* (2013). A decrease in the mean length of 1st and 2nd intercolar, 3rd and 4th leaf along with a significant reduction in the fresh and dry weight of the roots and shoots indicate a strong negative correlation between these parameters and NaCl stress. However, among all genotypes, a relatively less decline in overall growth of KS-282 suggest a better ionic/osmotic tolerance and survival rate (70%) which is similar to the field test conducted on the same genotype by Mumtaz *et al.* (2018). On the contrary, our results categorise Super Basmati as a highly sensitive genotype (78% plant mortality rate). KSK-133, Shaheen Basmati and DilRosh exhibited a moderate level of tolerance to 100 m*M* NaCl (65, 48 and 62% survival rate).

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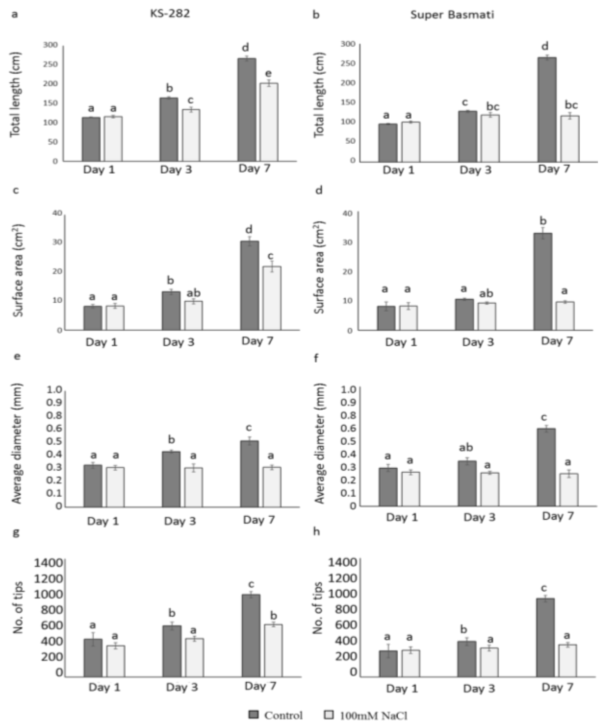

Fig. 5: Effect of NaCl on root development. Change in the total root length (a & b), surface area (b &c), diameter (d & e) and number of tips (g & h) of KS-282 and Super Basmati respectively under 100 m*M* NaCl stress. Different letters indicate statistically different expression levels according to two-way ANOVA ($p<0.05$) followed by post hoc Fisher's LSD test

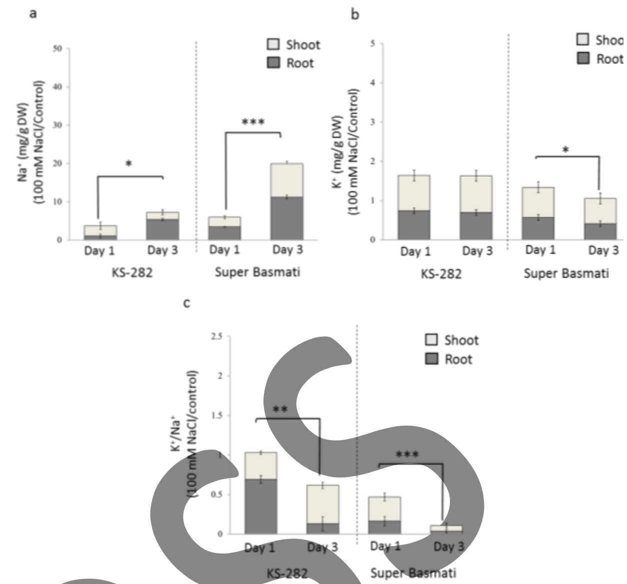

Fig. 6: The concentration of Na^+ and K^+ (mg/g dry weight) in the roots and shoots of contrasting genotypes. The Na^+ concentration in the roots and shoots at day 1 (a) and day 3 (b) and the K^+ concentration at day 1 (c) and day 3 (d) of salt treatment in KS-282 and Super Basmati. In the tolerant genotype, KS-282, Na^+ were restricted in the roots and a low level was evident in the shoots while a high and even distribution of Na^+ was observed in the roots and shoots of the sensitive genotype. Similarly, a higher K^+ content was observed in the roots and shoots of the tolerant plants as compared to the sensitive plants (c and d). Different letters indicate statistically different expression levels according to two-way ANOVA ($p<0.05$) followed by post hoc Fisher's LSD

Similarly, greater water loss through transpirational flow was evident in Super Basmati and Shaheen Basmati owing to a higher degree of stomatal conductance and resulting less relative water content. On the other hand, high water conservation was evident in KS-282 plants.

Root morphology analysis of the contrasting genotypes *i.e*., KS-282 and Super Basmati using WinRhizo pro image analysis system presented differences in root architecture and dynamics in response to salt stress, which can be correlated with the hydraulic conductivity and water conservation. Rice roots are characterized as fibrous and fine with an average diameter less than 0.5 mm (Gu *et al.,* 2017). Rice root system consists of a central nodal root having the greatest diameter and serves as the main pathway for the flow of water and nutrients to the areal parts. Lateral roots emerge from the nodal root and branch further to form a new higher lateral root system which forms the main site of water and nutrient absorption (Robin and Saha, 2015).

Many groups have analysed root morphology in rice, maize and barley (Gu *et al.,* 2017) but to our knowledge no data are available, which show changes in root dynamics in hydroponic media under salt stress using WinRhizo Pro software program. We found differences in the total root length, mean surface area, average diameter and total number of tips within the first three days of salt stress (Fig. 5a–h). KS-282 plants were able to reduce root density by decreasing root architectural parameters to conserve resources under osmotic and ionic stress during early phase of salt treatment as compared to the sensitive Super Basmati plants. In KS-282 plants, a decrease in the length of nodal and lateral roots was evident under NaCl stress while no such significant change was recorded in the roots of sensitive plants. Our data on total root length in response to salt stress is consistent to the previous studies on wheat (Shahzad *et al.,* 2012) and Arabidopsis (Brussens *et al*., 2000) while in contrast to results in chickpea (Boominathan *et al*., 2004) and later in Arabidopsis by He *et al*. (2005) who demonstrated an increase in the lateral root mass with high levels of NaCl to combat root hydraulic impairment (Krishnamurthy *et al.,* 2011).

Generally, it is believed that deeper, thicker and more branched root system can enhance drought tolerance in glycophytes (Gowda *et al*., 2011). On the contrary, we were able to see an early reduction in length, surface area, average diameter and number of tips in salt treated tolerant KS-282

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plants while no such reduction was observed in sensitive plants at day 3 of salt treatment (Fig.5a–h). Similar results have been reported by Rogers *et al*. (2016) through X-ray computed tomography of rice roots. Early reduction in the mean diameter can be linked with a reduced risk of xylem cavitation (Gowda *et al.*, 2011). Reduction in the surface area suggests reduced lateral root density while less number of tips specifies a significant reduction in meristematic, elongation and water absorption zones. A similar decrease in length and biomass has been reported in roots of barley (Shabala *et al.*, 2003) *Arabidopsis thaliana* (Wang *et al.*, 2008) and rice (Gu *et al*., 2017) under water stress. This reduction in lateral root density and consequent surface area may be of adaptive nature since fine lateral roots are easy to wither, particularly under drought conditions (Kawada, 1984). In contrast the sensitive Super Basmati plants did not show any change in root morphology as compared to the control plants for the first three days of salt treatment and hence a higher hydraulic conductivity and dissolved ion flow to the areal parts during the early phase of salt stress. These differences in root growth between the sensitive and tolerant genotypes may be correlated with different nutrient uptake levels, water and ion flow from the roots to the shoots and hence a variable Na^+ and K^+ ion content of plant body (Fig. 6a, b, c and d). After an initial delay in root growth, the roots of tolerant KS-282 sustained a gradual increase and recovery in selected parameters while root growth and development was strongly inhibited in the sensitive genotype together with an initiation of plant mortality owing to its failure to resist Na^+ intoxication. Similar results on root growth were obtained in rice salt sensitive3 (*rss3*) mutant by Toda *et al.* (2013).

Apoplastic ionic balance is altered greatly under saline stress (Krishnamurthy *et al.*, 2011) ultimately affecting turgor pressure. Our results demonstrate a rapid increase in Na^+ content in the roots and shoots of both genotypes after salt exposure (Fig. 6a and b). However, in KS-282, the increase of Na^+ in the roots and shoots was significantly low as compared to Super Basmati plants. Na^+ ion retention in roots (Fig. 6b) and a higher K^+ concentration in the roots and shoots (Fig. 6c and d) suggests a high K^+/Na^+ ratio in tolerant plants proposing a strict control over Na^+ extrusion, sequestration and xylem loading. A quick reduction in plant growth parameters (root and shoot biomass), higher water conservation, root growth delay along with K^+ and Na^+ homeostasis presented a strong correlation with plant survival rate under NaCl stress.

Conclusion

KS-282 genotype classified as salt tolerant, showed consistently lower root growth rates and surface area, more stable water content, less transpiration rate through a quick response of stomatal guard cells and better adaptation due to an early response of roots to salt stress. On the contrary sensitive Super Basmati plants failed to make these adaptive changes at root and shoot level. Rapid changes in the root architecture may confer the ability to regulate water and ion uptake to allow an efficient control of plant water status under salt stress.

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References

AbdElgawad, H., G. Zinta, M.M. Hegab, R. Pandey, H. Asard and W. Abuelsoud, 2016. High salinity induces different oxidative stress and antioxidant responses in maize seedlings organs. *Front. Plant Sci.*, 7: 276

Akram, S., M.N. Siddiqui, B.M. Hussain, M.A. Bari, M.G. Mosofa, M.A. Hossain and L.S.P. Tran, 2017. Exogenous glutathione modulates salinity tolerance of soybean [*Glycine max* (L.) Merrill] at reproductive stage. *J. Plant Growth Regul.*, 36: 877–888

Barr, H.D. and P.E. Weatherley, 1962. A re-examination of the relative turgidity technique for estimating water deficit in leaves. *Aust. J. Biol. Sci.*, 15: 413–428

Boominathan, P., R. Shukla, K. Kumar, D. Manna, D. Negi, P.K. Verma and D. Chattopadhyay, 2004. Long term transcript accumulation during the development of dehydration adaptation in *Cicer arietinum*. *Plant Physiol.*, 135: 1608–1620

Brussens, S., K. Himanen, B. van de Cotte, T. Beeckman, M. Van Montagu, D. Inze and N. Verbruggen, 2000. Expression of cell cycle regulatory genes and morphological alterations in response to salt stress in *Arabidopsis thaliana*. *Planta*, 211: 98–104

Fitter, A., 2002. *Characteristics and Functions of Root Systems*, 3rd edition, pp: 15–32. Plant Roots: The Hidden Half, Marcel Dekker Inc., New York, USA

Formentin, E., C. Sudiro, G. Perin, S. Riccadonna, E. Barizza, E. Baldoni, P. Lavezzo, P. Stevanato, G.A. Sacchi, P. Fontana, S. Toppo, T. Morosinotto, M. Zottini and F. Lo Schiavo, 2018.Transcriptome and cell physiological analyses in different rice cultivars provide new insights into adaptive and salinity stress responses. *Front. Plant Sci*., 9: 204

Gowda, V.R.P., A. Henry, A. Yamauchi, H.E. Shashidhar and R. Serraj, 2011. Root biology and genetic improvement for drought avoidance in rice. *Field Crops Res.*, 122: 1–13

Gu, D., F. Zhen, D.B. Hannaway, Y. Zhu, L. Liu, W. Cao and L. Tang, 2017. Quantitative classification of rice (*Oryza sativa* L.) root length and diameter using image analysis. *PloS One*, 12: e0169968

He, X.J., R.L. Mu, W.H. Cao, Z.G. Zhang, J.S. Zhang and S.Y. Chen, 2005. AtNAC2, a transcription factor downstream of ethylene and auxin signaling pathways, is involved in salt stress response and lateral root development. *Plant J.*, 44: 903–916

Julkowska, M.M., H.C.J. Hoefsloot, S. Mol, R. Feron, G.J. de Boer, M.A. Haring and C. Testerink, 2014. Capturing arabidopsis root architecture dynamics with root-fit reveals diversity in responses to salinity. *Plant Physiol.*, 166: 1387–1402

Kano, M., Y. Inukai, H. Kitano and A. Yamauchi, 2011. Root plasticity as the key root trait for adaptation to various intensities of drought stress in rice. *Plant Soil*, 342: 117–128

Kawada, S., 1984. *Root System of Rice*. Agriculture Press, Beijing, China

Krishnamurthy, P., R. Kosala, N. Shraddha, S. Lukas and M.K. Mathew, 2011. Root apoplastic barriers block Na^+ transport to shoots in rice (*Oryza sativa* L.) *J. Exp. Bot.*, 62: 4215–4228

In Press

Li, Y.H., 1979. *Morphological and Anatomy of Gramineous Crop*. Shanghai Scientific and Technical Publishers, Shanghai, China

Lutts, S., J.M. Kinet and J. Bouharmont, 1995. Changes in plant response to NaCl during development of rice (*Oryza sativa* L.) varieties differing in salinity resistance. *J. Exp. Bot*., 46: 1843–1852

Mumtaz, Z.M., S. Muhammad, G. Abbas, A. Javaid and Q. Zia, 2018. Genotypic variation in rice for grain yield and quality as affected by salt-affected field conditions. *J. Plant Nutr.*, 41: 233–242

Rao, P.S., B. Mishra and S.R. Gupta, 2013. Effects of soil salinity and alkalinity on grain quality of tolerant, semi-tolerant and sensitive rice genotypes. *Rice Sci*., 20: 284–291

Robin, A.H.K. and P.S. Saha, 2015. Morphology of lateral roots of twelve rice cultivars of Bangladesh: dimension increase and diameter reduction in progressive root branching at the vegetative stage. *Plant Root*, 9: 34–42

Rogers, E.D., D. Monaenkova, M. Mijar, A. Nori, D.I. Goldman and P.N. Benfey, 2016. X-ray computed tomography reveals the response of root system architecture to soil texture. *Plant Physiol*., 171: 2028–2040

Shabala, S., L. Shabala and E. Van Volkenburgh, 2003. Effect of calcium on root development and root ion fluxes in salinized barley seedlings. *Funct. Plant Biol*., 30: 507–514

Shahzad, A., M. Ahmad, M. Iqbal, I. Ahmed and G. Ali, 2012. Evaluation of wheat landrace genotypes for salinity tolerance at vegetative stage by using morphological and molecular markers. *Gene. Mol. Res*., 11: 679–692

Toda, Y., M. Tanaka, D. Ogawa, K. Kurata, K. Kurotani, Y. Habu, T. Ando, K. Sugimoto, N. Mitsuda, E. Katoh, K. Abe, A. Miyao, H. Hirochika, T. Hattori and S. Takeda, 2013. Rice salt sensitive3 forms a ternary complex with JAZ and class-C bHLH factors and regulates jasmonate-induced gene expression and root cell elongation. *Plant Cell*, 25: 1709–1725

Wang, H., J. Siopongco, L.J. Wade and A. Yamauchi, 2009. Fractal analysis on root systems of rice plants in response to drought stress. *Environ. Exp. Bot*., 65: 338–344

Wang, J., X. Zhang and C. Wu, 2015. Advances in experimental methods for root system architecture and root development. *J. For. Res*., 26: 23–32

Wang, Y., W. Zhang, K. Li, F. Sun, C. Han, Y. Wang and X. Li, 2008. Salt induced plasticity of root hair development is caused by ion disequilibrium in *Arabidopsis thaliana*. *J. Plant Res.*, 121: 87–96

Yeo, B.P.H., M. Bhave and S.S. Hwang, 2018. Effects of acute salt stress on modulation of gene expression in a Malaysian salt-tolerant indigenous rice variety, Bajong. *J. Plant. Res*., 131: 191–202

Zeng, L. and M.C. Shannon, 2000. Salinity effects on the seedling growth and yield components of rice. *Crop Sci.*, 40: 996–1003

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