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Original scientific article

SALT STRESS TOLERANCE POTENTIAL OF SELECTED RICE CULTIVARS OF PAKISTAN

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Tawab F., A. Ahmad, A. Nasim, S. Tawab, I. Munir (2021). *Salt stress tolerance potential of selected rice cultivars of Pakistan*. - Genetika, vol 53, no.3, 1093-1103.
Ten local rice varieties (Sarshar, Pakhal, Sada Hayat, Jajai-77, NIR-9, RI-DR-92, JP-5, Shaheen Basmati, Shadab-31 and TN-1) were evaluated for salinity stress (0, 50, 75 and 100 mM of NaCl) tolerance at seedling stage, in Completely Randomized Design (CRD). On an average the root length, shoot length and the relative growth rate of all rice varieties decreased linearly with increased levels of salinity. Based on the individual results of all the parameters studied, Pakhal was found to be the most salt tolerant variety. The germination rate of the varieties RI-DR-92 and TN-1 was better at higher levels of salinity (75 and 100 mM NaCl) among all the tested varieties. While, considerable reduction in germination rate was recorded for Jajai-77. The varieties RI-DR-92 and TN-1 that were more affected showing more reduction in shoot length (4.2 cm and 4.4 cm), root length (2.9 and 3.2 cm) and relative growth rate (0.22 and 0.14 g/gday), grown at 75 and 100 mM NaCl respectively. The variety Pakhal showed less reduction in shoot length (1.5 and 2.7 cm), root length (0.95 and 1.0 cm) and relative growth rate (0.073 and 0.075 g/gday), grown at 75 and 100 mM NaCl, respectively as compared to control. However, according to Stress Susceptibility Index (SSI), TN-1 was observed to be the most tolerant and Jajai-77 was the most susceptible variety to salt stress. The most tolerant rice varieties differentiated in the current study could be used to produce higher yields under saline conditions.
Keywords: Salinity, Stress Tolerance, Rice, Cultivars, Seedling stage

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INTRODUCTION

Rice (*Oryza sativa L.*) is a significant major staple food throughout the world, as it is used to nourish almost 50% of the people around the globe (KHUSH, 2005). On the basis of its nutritional value, rice is planted over 150 million hectares of land annually, covering about 11% of the world's arable land (CHAKRAVARTHI and NARAVANENI, 2006). In Pakistan, Rice is an important food as well as cash crop. It is the second main staple food crop after wheat and the second major exportable commodity after cotton. It contributes 3.5 percent of value added in agriculture and 0.7 percent in GDP (ANONYMOUS, 2021). Unfortunately soil salinity has become a severe menace to agricultural productivity, especially in infertile and semiarid parts of the world (MUNNS, 2002). Along with wheat, maize and barley rice yield is declined up to 70% due to salinity (ACQUAAH, 2007). It is therefore crucial to develop salt tolerant food and fiber crops to meet the demand of agriculture productivity in salt affected areas. (ROZEMA and FLOWERS, 2008; JOSHI *et al.*, 2015).

Salinity restricts agriculture preventing crop productivity all around the globe including Pakistan (ASHRAF and FOOLAD, 2007; MEHMOOD *et al.*, 2009). In Pakistan, because of salinity 40,000 ha of cultivated land is wasted annually (AHMAD *et al.*, 2006; ASHRAF *et al*., 2008; MEHMOOD *et al.*, 2009). High salinity has been found to affect many physiological processes, i.e., photosynthesis, respiration, nitrogen fixation and carbohydrate metabolism (CHEN *et al.*, 2008).

The extent of tolerance in rice to salinity changes with the plant stage, seedling and flowering stages are considered vital (SINGH and FLOWERS, 2010). However, depending on the genotype and the crop cycle, plant when exposed to salinity is highly affected at vegetative stage than at the flowering stage (ZENG *et al.*, 2001). The biomass production is harmed by increase in salinity (ZENG *et al.*, 2001; MELO *et al.*, 2006; CARMONA *et al.*, 2009). One of the effective methods to increase the productivity of saline soil is to identify a salt tolerant cultivar. Salt tolerance is a multigenic attribute that allows the plants to grow and maintain profitable yield in the presence of non-physiologically high and constant level of salt, particularly NaCl. Therefore, raising crops tolerant to saline environments is crucially needed to facilitate agriculture in marginal lands. Efforts on molecular, biochemical and physiological levels are employed in order to raise rice plant with enhanced aptness towards varying environmental stresses. The objective of this study was to evaluate local rice varieties of Pakistan for their stress tolerance potential using some of the physiological parameters.

MATERIALS AND METHODS

Plant materials and growth condition

In total ten rice varieties were used in this research project, that were kindly provided by the Department of Plant Breeding and Genetics, University of Agriculture, Peshawar. These varieties included Sarshar, Sada Hayat, Jajai-77, NIR-9, JP-5, RI-DR-92, Shaheen Basmati (S.B), Pakhal, Shadab-31 (S-31) and TN-1.

Two types of media were used for the growth of plants under *in vitro* condition. Only half strength media used as control and half strength MS media supplemented with different concentrations of NaCl. To make 1M NaCl solution 5.85 g of NaCl was dissolved in 100 mL of distilled water, and by using the molarity formula, 1M NaCl solution was diluted to 25, 50, 75 and 100 mM.

Germination percentage

For germination experiments fifteen seeds of each variety were surface sterilized and sprinkled in 1 mL of water which was used as a control and salt solutions with different concentrations (25, 50, 75 and 100 Mm NaCl), and the germination of seeds of each variety was checked within two days of incubation at 24 ± 1°C. After five days the water and the salt solutions were supplemented with half strength MS media and the number of seeds germinated in each Petri dish recorded daily for 8 days. Germination recorded at 25 mM NaCl was omitted due to nearly same response as control.

Measurement of growth parameters

The plants grown in water and salt solutions were analyzed for some of physiological parameters including root length, shoot length root/shoot length ratio and relative growth rate at (25, 50, 75 and 100 mM NaCl). Root and shoot lengths were determined after 12 days and the data obtained was compared graphically. Root/Shoot length ratio was determined for all the ten genotypes under control and salt stress conditions and the data obtained was compared graphically.

Relative growth rate (0, 75 and 100 mM NaCl) of all the ten genotypes were calculated by weighing the three week old plants. These plantlets were then transferred to hydroponic boxes filled with half strength MS media with different concentration of salt. For differentiation of control and salt stress samples, each plantlet was tagged. After 7 days of treatment the plants were weighed again. The same experiment was repeated three times. The relative growth rate (RGR) was calculated using the equation described by POORTER and GARNIER (1996) and stress susceptibility index (SSI) was calculated in order to find the most tolerant varieties by following FISCHER and MAURER (1978). The data for all the parameters was analyzed using the two-factorial Completely Randomized Design (CRD).

RESULTS

Seed germination

Highly significant differences ($P \leq 0.01$) were observed for germination rate among different levels of salinity and varieties and their interaction. Seed germination rate varied among all the ten varieties at control condition from 33 to 100%. NIR-9 and S-31 showed highest germination rate (100%) while Shaheen basmati showed the lowest percentage of germination (33%).

Overall, the germination rate of all the ten varieties showed negative trend with increase in salt concentrations (50, 75 and 100 mM NaCl). At 50 mM NaCl highest reduction in germination rate was observed in genotype Jajai-77 which was (15.5%) reduced as compared to control. However, the genotype NIR-9 showed lowest reduction (4.4%) as compared to control, followed by genotypes RI-DR-92, JP-5 and S-31 (6.6%). At 75 mM NaCl the genotype Jajai-77 showed highest reduction (26%) in germination rate as compared to control while the lowest reduction (11.1%) was recorded for the genotypes RI-DR-92 and TN-1. The lowest reduction in germination rate was observed for genotypes RI-DR-92 and TN-1 grown on highest level of salinity i.e.100 mM NaCl as compared to their controls. The highest reduction was again observed for the genotype Jajai-77 which was (33%) less as compared to control as shown in Figure1. The germination rate of the genotypes RI-DR-92 and TN-1 was best on higher levels of salinity (75 and 100 mM NaCl) among all the ten varieties used under study.

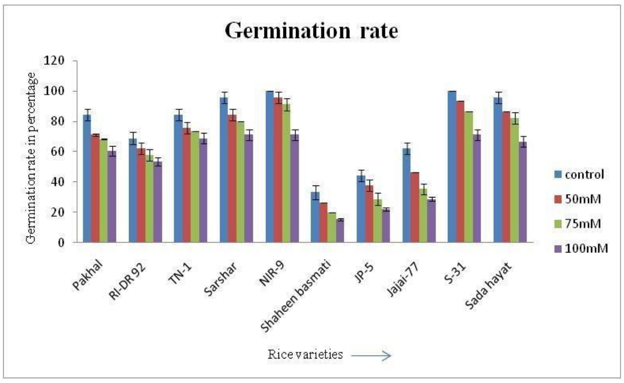

Figure 1. Changes in germination rate of rice seeds under control and NaCl (50, 75 and 100 mM) stress. Values are averages ± SE

Seedling shoot length

Highly significant differences (P≤0.01) were observed for seedling shoot length among different levels of salinity and varieties and their interaction was found to be non significant. All the ten varieties showed different shoot lengths under control conditions ranging from (3.8 to 6.7 cm). Highest shoot length was recorded for genotype TN-1 and lowest for genotype Shaheen Basmati which was (6.7 and 3.8 cm), respectively.

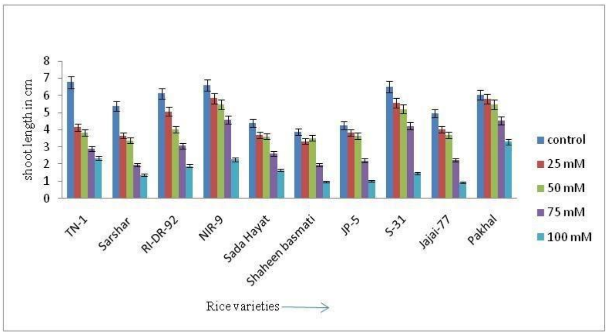

Figure 2. Shoot lengths of rice plants grown under control and different concentration of salt solutions (25, 50, 75 and 100 mM NaCl). Values are averages ± SE

On the whole salt stress had reducing effect on shoot lengths of all genotypes under study. Plants grown in the nutrient solution with 25, 50, 75 and 100 mM salts were short in size than the plants grown under control condition. At 25 mM NaCl highest reduction in shoot length was recorded for genotype TN-1 which was (2.6 cm) shorter than control. However, lowest reduction (0.2 cm) in soot length was observed for the genotype Pakhal, followed by the variety JP-5 which was (0.4 cm) shorter as compared to control. At 50 mM NaCl, highest reduction (2.9 cm) in shoot length as compared to that of control was recorded for the genotype TN-1, followed by the variety RI-DR-92 which showed (2.1 cm) less shoot length than its control. However, the lowest reduction (0.3 cm) in shoot length was recorded for the genotype Shaheen Basmati followed by Pakhal (0.5 cm) as compared to their controls. The highest reduction (3.8 cm) in shoot length was again recorded for the genotype TN-1 grown at 75 mM NaCl while the lowest reduction (1.5 cm) was observed for the variety Pakhal. At 100 mM NaCl, S-31 showed the highest reduction (5.0 cm) in shoot length as compared to control followed by the genotypes TN-1 and NIR-9 (4.4 and 4.3 cm), respectively. However the lowest reduction (2.7 cm) in shoot length was recorded for the genotypes Pakhal and Sada Hayat as shown in Figure 2. Pakhal and Sada Hayat were less affected by increased level of salinity i.e. (75 and 100 mM NaCl).

Seedling root length

Highly significant differences ($P \leq 0.01$) were observed for seedling root length among different levels of salinity and varieties and their interaction. At control condition, all the ten varieties showed different root lengths ranging from (1.6 to 3.8 cm). Highest root length (3.8 cm) was recorded for genotype Pakhal and lowest (1.6 cm) for genotype Shaheen Basmati. Highest reduction (0.77 cm) was recorded for genotype Sarshar grown at 25 mM NaCl while the lowest reduction (0.39 cm) as compared to control was shown by genotype JP-5. NIR-9 was the only variety that showed more root length at 25 mM NaCl as compared to that of control.

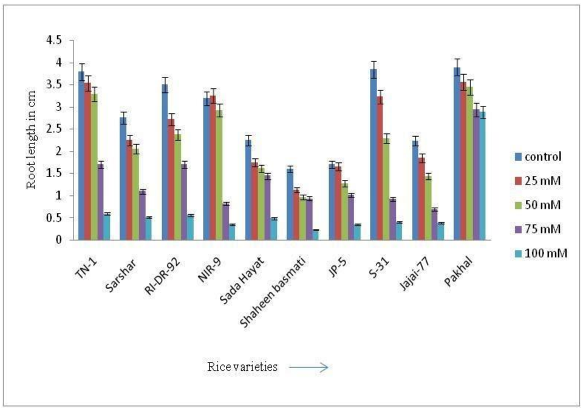

Figure 3. Root lengths of rice plants grown under control and different concentration of salt solutions (25, 50, 75 and 100 mM NaCl). Values are averages ± SE

At 50 mM NaCl highest reduction in root length was observed for the genotype S-31, followed by RI-DR-92 which was (1.5 and 1.1 cm) less as compared to their controls. However, the lowest reduction (0.2 and 0.4 cm) was recorded for the genotypes NIR-9 and Pakhal, respectively. At 75 mM NaCl highest reduction in root length was observed for the variety NIR-9, followed by TN-1 which was (2.3 and 2.0 cm) less as compared to control. However, the lowest reduction (0.68 cm) was recorded for the genotype JP-5. The genotype S-31 was affected more at 100 mM NaCl whose root length was reduced (3.4 cm) as compared to all the genotypes, followed by the variety TN-1 which showed (3.2 cm) reduction in root length as compared to its control. The lowest reduction (1.01 cm) was recorded for the genotypes Pakhal, followed by Shaheen Basmati and JP-5 which showed less reduction (1.3 cm) as compared to their control as shown in Figure 3.

NIR-9 was the only variety showing less root length at control but more root length at 25mM NaCl. NIR-9 was less affected at 50 mM NaCl as compared to all the ten genotypes used under study. At 75 mM NaCl the lowest reduction in root length was observed for Sada Hayat and at 100 mM NaCl for the genotype Pakhal.

Root/shoot length ratio

Significant differences (P≤0.05) were observed for seedling root/shoot length among different levels of salinity and varieties and their interaction was found to be non-significant. At control condition the highest root/shoot ratio was recorded for the genotype Pakhal while the lowest was recorded for JP-5 followed by Shaheen Basmati. At initial stage of stress (25 and 50 mM NaCl) an increase in root/shoot ratio as compared to that of control was observed for most of the genotypes i.e. TN-1, Sarshar, NIR-9 and JP-5. However, at 75 mM NaCl the highest root/shoot ratio was observed for the genotype Pakhal and the lowest was recorded for NIR-9, followed by S-31. At 100 mM NaCl the highest ratio was again recorded for Pakhal while the lowest was observed for the genotype NIR-9, followed by TN-1 as shown in the Figure 4.

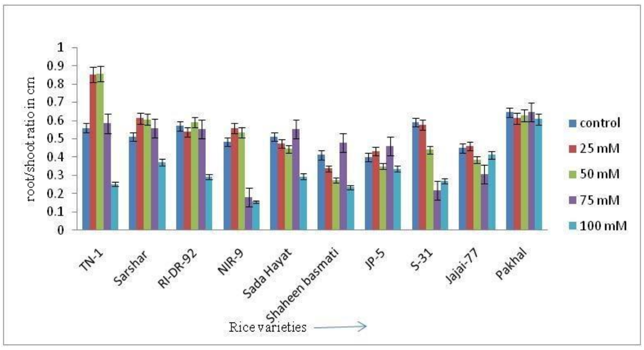

Figure 4. Root/shoot ratio of rice plants grown under control and different concentration of salt solutions (25, 50, 75 and 100 mM NaCl). Values are averages ± SE

Relative growth rate (RGR)

Highly significant differences (P≤0.01) were observed for seedling root/shoot length among different levels of salinity and varieties and their interaction. All the ten varieties were analyzed for relative growth rate at control and 75 and 100 mM of salt stress conditions. In general, all the plants showed different RGR grown in control conditions. At control condition, highest RGR was observed for genotype RI-DR-92 while the lowest was observed for genotype JP-5. At 75 mM NaCl highest reduction (0.12 g/g day) in RGR was observed for the genotype RI-DR-92. However, the lowest difference (0.03 g/g day) with that of control was recorded for the genotypes JP-5 and Jajai-77 followed by the genotypes Pakhal, Sada Hayat and NIR-9 whose RGR was recorded (0.07 g/g day) less as compared to that of control. At 100 mM NaCl highest reduction (0.33 g/g day) in relative growth rate was recorded for the genotype Sarshar, while the lowest (0.07 g/g day) was observed for the variety Pakhal. RGR is represented graphically in Figure 5.

The genotype RI-DR-92 showed highest RGR at control while at 75 mM NaCl the genotypes TN-1, JP-5 and Jajai-77 were less affected as compared to their control. Pakhal was observed to be the best variety that was less affected by high salt stress (100 mM NaCl) as compared to that of control.

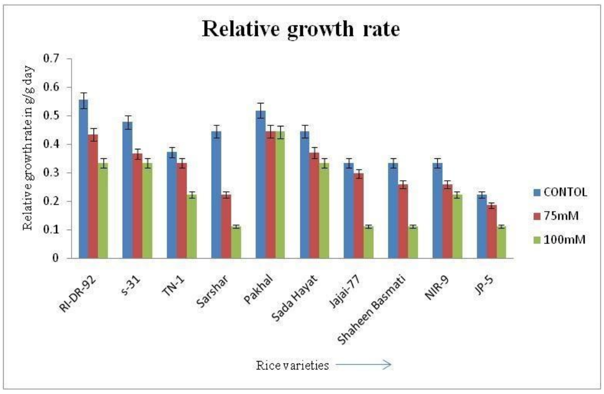

Figure 5. RGR of rice seedlings grown under control and 75 and 100 mM NaCl stress.Values are averages ± SE

Stress susceptibility index

Stress susceptibility index (SSI) is the relative loss of a genotype because of exposure to stress compared with mean loss of all the genotypes. The mean performance of the genotypes is statistically different significantly, thus the SSI was calculated to identify the most tolerant varieties on the basis of germination and relative growth rate at 75 and 100 mM NaCl concentrations. At salt concentration of 75mM NaCl, the SSI for germination ranged between 0.5 and 2.0 Figure 6A. The germination of NIR-9 was least affected by salt stress (SSI = 0.5), followed by TN-1 (SSI = 0.7). On the other hand, Jaijai-7 was the most susceptible variety to salt stress (SSI = 2.0). As a whole, six varieties were more tolerant than pakhal (SSI = 1.0). Furthermore, as expected the germination was affected to a greater extent with increasing salt concentration (SSI = 1.2 – 3.6) Figure 6B. At 100mM TN-1 was the least affected variety (SSI = 1.2) by the salt stress, followed by RI-DR-92 (SSI = 1.5), whereas Jaijai-7 and Shaheen basmati were the most susceptible varieties (SSI = 3.6). Similarly, three varieties were more tolerant at 100mM salt stress than the variety Pakhal (SSI = 1.9) as shown in Figure 6.

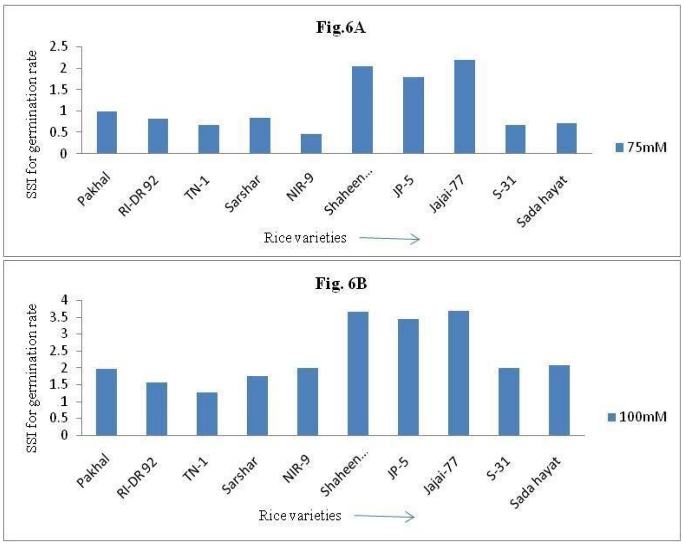

Figure 6A and B. Stress susceptibility index for germination rate of different rice varieties at 75 mM and 100 mM NaCl concentrations

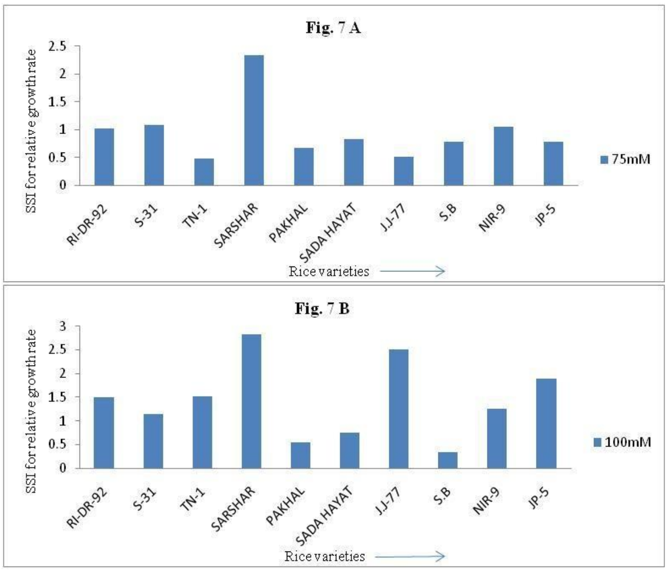

Figure 7A and B. Stress susceptibility index for RGR of different rice varieties at 75mM and 100mM NaCl concentrations

Similarly, at 75 mM NaCl, the SSI for relative growth rate ranged between 0.5 and 2.0 Figure 7A. The RGR of TN-1 was least affected by salt stress (SSI= 0.5), followed by the genotype Jajai-77 (SSI= 0.6).

However, the variety Sarshar was observed to be the most susceptible to salt stress (SSI=2.0). At higher salt concentration i.e. at 100 mM NaCl Shaheen Basmati was the least affected variety (SSI=0.4). However, Jajai-77 and Sarshar were more susceptible to salt stress (SSI= 2.0) as shown in the Figure 7B.It is concluded that TN-1 is the most tolerant variety while Jajai-77 is the most susceptible variety among all the ten varieties used under study based on the SSI calculated for germination and RGR.

DISCUSSION

Rice production is severely hampered by salinity. There are many approaches to identify rice varieties for their stress tolerance potential. The present study was carried out with the aim to identify and differentiate local rice verities of Khyber Pakhtunkhwa for their salt stress tolerance potential by studying some of the physiological parameters at early seedling stage. The results of present study demonstrated that the NaCl induced salt stress significantly influenced the germination rate of different local rice varieties when expose to an increase in salinity level from 0 to 100mM NaCl. This is in agreement with (DUAN *et al.,* 2004), who also found that rice growth is highly sensitive to salinity at germination and early seedling stage as these stages are considerably affected by osmotic stress and specific ion toxicity. Similarly the shoot length, root length and relative growth rate of the treated plants decreased as compared to the non-treated ones. Similar results have been obtained by (ALAM *et al.,* 2004), who worked on indica rice varieties treated with 0.0, 4.5, 8.5 or 12.5 dS m-1 (approximately equivalent to 0, 45, 85 and 125 mM NaCl, respectively). It is therefore concluded that the severity of salinity negatively affected the germination and growth of plant as shown by the rate of germination, shoot length, root length and relative growth rate.

Exceedingly high dose of salinity ultimately results in death of plants however moderate salt stress results in differences in growth among different crop varieties. The mean performance of the genotypes was statistically different significantly thus the SSI was calculated to identify the most tolerant varieties on the basis of germination and relative growth rate at 75 and 100mM NaCl concentrations. It was concluded that the genotype TN-1 was good both at germination as well as relative growth rate and the variety Jajai-77 was observed to be the most susceptible variety among all the ten genotypes used under study.

Salinity affects morphological features of various rice varieties but their response varies with stages of the plant growth. TN-1 and RI-DR-92 were better at germination than Pakhal but less responsive at seedling stage. Based on individual physiological experiments, Pakhal was concluded to be the tolerant variety as least affected by high salinity. However, according to the stress susceptibility index (SSI), TN-1 was observed to be the more tolerant variety and Jajai-77 was found to be the more susceptible variety. These differences may be due to genetic diversity and inherited differences among the genotypes as different cultivars behave differently under saline conditions.

CONCLUSIONS

Based on our results, we conclude that among all the tested varieties for salt stress tolerance potential genotype Pakhal showed better performance as compared to all other genotypes based on individual physiological parameters. However, TN-1 was observed to be the most tolerant variety on the basis of SSI index.

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TOLERANCIJA NA STRES SOLI KOD KULTIVARA PIRINČA U PAKISTANU

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Izvod

Deset lokalnih sorti pirinča (Sarshar, Pakhal, Sada Hayat, Jajai-77, NIR-9, RI-DR-92, JP-5, Shaheen Basmati, Shadab-31 and TN-1) ocenjeni su na tolerantnost na stres soli (0, 50, 75 and 100 mM of NaCl) u fazi klijanaca, u kompletno randomiziranom dizajnu (CRD). U proseku dužina korena, dužina izdanaka i relativna stopa rasta svih sorti pirinča opadali su linearno sa povećanjem nivoa slanosti. Na osnovu pojedinačnih rezultata svih proučavanih parametara, utvrđeno je da je Pakhal sorta koja najviše podnosi salinitet. Klijavost sorti RI-DR-92 i TN-1 bila je bolja na višim nivoima saliniteta (75 i 100 mM NaCl) među svim ispitivanim sortama, dok je kod Jajai-77 zabeleženo značajno smanjenje klijanja. Sorte RI-DR-92 i TN-1 koje su bile više pogođene pokazuju veće smanjenje dužine izdanaka (4,2 cm i 4,4 cm), dužine korena (2,9 i 3,2 cm) i relativne brzine rasta (0,22 i 0,14 g/gdan), uzgajane na 75 i 100 mM NaCl respektivno. Sorta Pakhal pokazala je manje smanjenje dužine izdanaka (1,5 i 2,7 cm), dužine korena (0,95 i 1,0 cm) i relativne brzine rasta (0,073 i 0,075 g/gdan), uzgajane na 75 i 100 mM NaCl, respektivno u poređenju sa kontrolom. Međutim, prema Indeksu osetljivosti na stres (SSI), primećeno je da je TN-1 najtolerantniji, a Jajai-77 najpodložnija vrsta stresu soli. Najtolerantnije sorte pirinča diferencirane u ovoj studiji mogu se koristiti za proizvodnju većih prinosa u fiziološkim uslovima.

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