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Performance of fifteen rice (*Oryza sativa* L.) cultivars in relation to early seedling growth traits against NaCl salinized water

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Abstract:

A laboratory experiment was conducted with the objectives to assess rice seedling growth performance and to examine the range of genetic variability for salinity tolerance among fifteen rice cultivars in a completely randomized design (CRD) with two factors and three replication at Seed Testing Laboratory, Department of Agronomy, Sindh Agriculture University Tandojam Pakistan under four salinity (control 00, 50, 100 and 150 mM L^{-1} NaCl) levels. Data were analyzed statistically for germination (%), shoot and root length, shoot fresh and dry weight, root fresh weight, root: shoot ratio and seedling vigour index. The results showed that rice seed germination percent, shoot, root length, shoot fresh and dry weight, root fresh weight, root:shoot ratio and seedling vigour index were significantly decreased in all cultivars due to increasing salinity level. The higher values of studied traits were obtained in control (00 mM) treatments. However, highest decline was observed at higher concentrations of 150 mM NaCl. In this

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study variation among the cultivars at different salt concentrations was noted. Cultivars IR-6 and DR-82 showed better performance for germination percent. DR-83, DR-82, Latify and IR-6 had longest shoots, roots, root:shoot ratio and seedling vigour index. Maximum shoot fresh weight was documented in DR-83, followed by IR-6. However cultivar Sada hayat and Mashoori remained more sensitive to salinity based on data of studied parameters. It is concluded that DR-82, DR-83, Latify and IR-6 might be used for further study of salinity effect on growth processes and its physiological consequences at an advanced stage of growth. These cultivars were more tolerant to salinity and recommended to use them in breeding program for enhancing rice production in Sindh.

Key words: Seeding germination, Rice varieties, Salinity tolerance

INTRODUCTION

Among the low and middle income countries of the world rice is the most important cereal crop. Its production in Pakistan holds an extremely important position in agriculture and the national economy [1]. Pakistan is the world's fourth largest producer of rice, after China, India and Indonesia. Most of these crops are grown in the fertile Sindh and Punjab region with millions of farmers relying on rice cultivation as their major source of employment. Among the most famous varieties grown in Pakistan include the Basmati, known for its flavor and quality.

Salinity is one of the major abiotic constraints on crop production and food security and adversely impact the social-economic fabric in many developing countries, affecting about 95 million hectares worldwide [2]. About UNEP (United Nations Environment Program) estimates that 20% of the agricultural land and 50% of the cropland in the world is salt-stressed [3]. Salinity is considered as one of important factors responsible for low rice production. It is the second most widespread soil problem in rice growing countries after drought and is considered as a serious constraint to increased rice production worldwide [4]. Various abiotic stresses including high or low temperature, water scarcity, high salinity and heavy metals exert drastic antagonistic effects on crop metabolism and thereby plant growth, development and ultimately crop productivity. Salinity is one of the most brutal environmental factors limiting the productivity of crop plants because most of the crop plants are sensitive to salinity caused by high concentrations of salts in the soil, and the area of land affected by it is increasing day by day [5].

Rice is sensitive to salinity particularly at the seedling stage. Screening/breeding of rice varieties for tolerance to salinity have been carried out for over three decades and various screening methodologies are used to screen out tolerant varieties [6]. Although salinity affects all stages of growth and development of rice, crop responses to salinity varies with growth stages, concentration and duration of exposure to salt [7] salinity at the reproductive stage depresses grain yield much more than salinity at the vegetative stages [6]. Rice is tolerant during germination, very sensitive during early seedling stage, tolerant during vegetative stage, again becomes sensitive during pollination and fertilization, and then becomes increasingly tolerant towards maturity[8]. Salt stress undesirably affects plant growth during all developmental stages, therefore, it is a major threat to crop productivity. The response to salinity varies with growth stages, varieties/ genotypes, and of salinization [9]. Several investigators have stated that salt exerts negative effect on seed germination and seedling growth [10]. Salt and osmotic stresses are responsible for both inhibition or delayed seed germination and seedling establishment [11]. The identification of genotypes having potential salt tolerance is an effective approach to solve the problems of saline soils [12]. This research study was planned to screening of rice varieties for tolerance to salinity at seedling stage.

MATERIALS AND METHODS

A laboratory experiment was conducted at Seed Testing Laboratory, Department of Agronomy, Sindh Agriculture University, Tandojam, Pakistan (25° 25′ 35.68″ N 68° 32′ 22.31″ E) under Completely Randomized Design (CRD) (factorial). Factor A, fifteen rice cultivars: C1= JJ-77, C2= DR-92, C3=Sada hayat, C4= DR-58, C5= IR-8, C6= Mashoory, C7= DR-62, C8= DR-6, C9= Shahkar, C10= DR-61, C11= Kanwal-95, C12= Latify, C13= DR-46, C14= DR-82 and C15= DR-83. Factor B, four salinity (NaCL *mM*) levels: S1= Distilled water (0 *mM*), S2= 50 *mM*, S3=100 *mM* and S4= 150 *mM*.

Planting material

The seeds of 15 rice cultivars were collected from Rice Research Institute (RRI) Dokri, Larkana, district of Sindh, Pakistan. They were surface sterilized with 0.5% sodium hypochloride solution (*v/v*) for 1 min, after sterilization, healthy and homogenous seeds of all varieties were washed thoroughly with distilled water. The seeds of each cultivar was allowed to germinate on double layer filter paper in 9 cm Petri dishes with three replications. To determine the effects of salinity; different concentrations of saline water (integrated as factor-A) was applied right from seed sowing to last day of germination. All the petri dishes were kept at 25 ± 1°C for 20 days.

Observations recorded

Germination (%) of the each rice cultivars, 20 seeds were placed in petri dishes for germination. The germinated seeds were counted after 20 days in the petri dishes. The germination was calculated through method given by Ellis and Robert [13] (1981) and [14] on 20th day of sowing: Germination = Number of germinated seeds /total number of seeds x 100. Shoot and root length (cm), the seedlings in each petri dish were used for measuring the shoot and root characteristics. After 20 days, the seedlings were separated into roots and shoots. The distances from the crown to leaf tip and root tip were measured as the shoot length and root length, respectively. Shoot and root fresh weight (g), the shoot and root fresh weights of each seedling were measured. The average fresh weights of shoot and root of each plant seedling were measured by dividing the total weight by the total number of seedlings. Seedling dry weight (g), same seedlings were used for measuring shoot dry weight after drying in oven at 68°C for 72 h. The seedlings were cooled at ambient temperature then weighing on digital top loading balance machine. Root/ shoot ratio, was calculated for weight by dividing root values by shoot values and seedling vigour index, seedling vigour index in each treatment was calculated as a Vigour index = Root length + Shoot length x Seed germination %.

Statistical analyses

All obtained data were statistically analyzed according to the technique of analysis of variance (ANOVA) for the factorial design using means of "MSTAT-C" computer software package. Least Significant Difference (LSD) method was used to test the differences between treatment means at 5% level of probability [15.

RESULTS

Final Germination Percentage (FGP)

Statistically significant ($P<0.05$) differences were observed among NaCl concentrates, rice cultivars and their interaction for final germination percentage. The percentage of germination significantly decreased in all cultivars due to increasing salinity and were inversely related to salt concentration level. (Table 1). In the control (0 mM L^{-1}), the germination percentages of all varieties were greater than 95%. Germination of rice seeds reached maximum (100%) in all fifteen cultivar sown in distilled water followed by (79.22%), seeds exposed to slightly saline concentration (50 mM). However, at higher concentrations of NaCl (100 and 150 mM) germination of plants was inhibited. Highest decline in germination (58.67%) was observed in petridishes exposed to highest salt concentration of 150 mM.

Salinity stress affected rice germination but to different extents in different cultivars. This study identified and compared salinity tolerance levels of 15 rice cultivar during germination (Table 2). Variation among the cultivars at different salt concentrations was noted. Its values were obtained between 69.58 % and 80.0% with 200 mM NaCl. The 100 and 150 mM NaCl concentrations led to significant differences among the varieties. Cultivars IR-6 and DR-82 showed better performance and did not differ significantly from each other with regard to final germination percent and, therefore, are considered as salt tolerant cultivars. Both these cultivars had germination percent higher than 63%, even with the 1500 mM NaCl concentration. For the interaction between salt concentration and cultivars, it was clear that Sada Hayat cultivar treated with 100 or 150 mM of NaCl gave the lowest percent of seed germination (50 and 69.58%, respectively).

Shoot fresh weight

Results shown in Table 1 showed that salinity levels, rice cultivars and their interaction was significant ($p<0.05$) for shoot fresh weight. Maximum shoot fresh weight was recorded in petridishes which received distilled water (control). However, shoot fresh weight was drastically decreased due to application of NaCl salinized water. Utmost decline in fresh weight was observed in rice cultivars at 100 (0.5173 g) and 150 mM (0.4189 g) NaCl.

In case of rice cultivars response against saline water, great variation was noted among them. Maximum shoot fresh weight was documented in DR-83, (0.70 g) followed by IR-6 (0.6867 g), whereas, DR-58 had minimum (0.5025 g) shoot fresh weight. The other ones were moderately affected by the salt treatment (Table 2).

Shoot dry weight

Data presented in Table 1 showed depressing effect of NaCl seeds saturated with 150 and 200 mM NaCl. Shoot dry weight showed inverse relationship with salt concentration. In the present study, shoot dry weights were reduced gradually with increasing NaCl concentrations from 0 to 150 mM. Highest rice shoot dry weight was calculated in treatment where seedlings were saturated with distilled water (control). Whereas, shoot dry weight was gradually reduced due to application of NaCl salinized water and hence, lowest fresh weight was noted in rice cultivars saturated with 150 mM (0.004222 g) NaCl.

Regarding salinity tolerance of 15 rice cultivars, (Table 2) a non significant differences were found against Na Cl levels for shoot dry weight. Among all tested rice cultivars, higher shoot dry weight values were recorded in DR-83. Whereas, Sada Hayat found to be more sensitive having lower shoot dry weight.

Root /shoot ratio

Statistically significant ($P<0.05$) difference were noted among salinity levels, rice cultivars and their interaction for root/ shoot ratio of rice cultivars. The root/ shoot ratio decreased in all cultivars due to increasing NaCl salinity (Table 1). In the control (00 mM L^{-1}) and 50 mM L^{-1} salinity level, the root/ shoot ratio of all varieties were found higher (1.252 and 1.245), whereas, it gradually decreased with increasing saline concentration and reached lowest ratio of 1.191at the highest salinity level (150 mM).

Salinity stress caused retardation in root/ shoot ratio to different extents in different cultivars. The 100 and 150 mM NaCl concentrations led to significant differences among the varieties. Cultivars DR-61 (1.386) showed better performance with maximum root/ shoot ratio. Whereas, IR-6 cultivar had minimum root/ shoot ratio of 1.139. Rest of cultivars were remained intermediate in terms of root shoot/ ratio (Table 2).

Seedling vigor index

There was a decreasing trend in vigor index as the salt concentration increased (Table 1). The analysis of variance indicated that increase in salinity significantly ($p<0.05$) decreased seedling vigor index. The highest vigor index of seedling were recorded on control treatment; and the lowest vigor indices were observed on 150 mM NaCl concentration. The interaction between varieties and salt concentration was significant.

In saline conditions, increasing salt concentration from 50 to 150 mM significantly decreased vigor index of all the tested rice cultivars (Table 2).The cultivars DR-82, DR-83, Latify and IR-6 showed more vigor index (1073, 1070, 1063 and 1062, respectively). However, less value of vigor index was obtained in cultivar DR-61 (920.7).

DISCUSSION

Seed germination and early seeding growth of rice has been shown to be highly sensitive to salt stress because of marked effects of osmotic stress and specific ion toxicity on these growth stages in this plant species [16]. The current results clearly showed rice seed germination %, shoot, root length, shoot fresh and dry weight, root-shoot ratio and seedling vigour index were significantly decreased in all cultivars due to increasing salinity and were inversely related to salt concentration level. The higher values of studied traits were obtained in control (0.0 *mM)* treatments. However, at higher concentrations of NaCl (100 and 150 *mM*) highest decline was observed in pertidishes. This result comes in consistent with previous ones [17,18]. [19] stated that the weight of shoots and roots showed high correlation with the level of salinity. [20] mentioned that the reduction in seedling fresh and dry weight is due to decreasing water uptake by seedlings in salt stress presence. [21] found a large variation in salinity tolerance among different rice germplasms. They stated that salt stress of 12 dSm^{-1} reduced seedling total dry matter by 40.6% of tolerant lines and by 46.0-73.5% for susceptible lines. Similar results were reported for corn [22]) and rice [23] at which relative salt sensitivity and tolerance differ among different cultivars. Elevated levels of salinity affected germination characters might be due to toxic effects of salinity stress. Salinity decreased germination typescripts of seeds which are directly related to the amount of absorbed water by the seed [24]. Seed germination was quantitative trait controlled by several gens and strongly affect by salt stress [25]. Salt stress undesirably affects plant growth during all developmental stages, therefore, it is a major threat to crop productivity. The response to salinity varies with growth stages, varieties/ genotypes, and of salinization [9]. Several investigators have stated that salt exerts negative effect on seed germination and seedling growth [26, 27, 10]. In present study salinity stress affected germination percent, shoot, root length, shoot fresh and dry weight, root-shoot ratio and seedling vigour index but to different extents in different cultivars. Cultivars IR-6 and DR-82 showed better performance for germination percent. Mean data showed that cultivars DR-

83, DR-82, Latify and IR-6 had longest shoots, roots, root shoot ratio and seedling vigour index. Maximum shoot fresh weight was documented in DR-83, followed by IR-6. However, non-significant higher values of shoot dry weight was observed in DR-83. Furthermore, cultivar Sada hayat and Mashoori remained more sensitive to salinity based on data of studied parameters. Salt tolerance is not a function of single organ or plant attribute, but it is the product of all the plant attribute [28]. Therefore a genotype exhibiting relative salt tolerance for all the plant attributes may be ideal one. It is evident from the results of present study that the EC level of 150 *mM* depressed the yield and yield components of all the cultivars under study. The only exception was DR-82, DR-83, IR- 6 and Latify where for most of the plant attributes little effect of salinity treatment was observed. The magnitude of reduction varied not only for plant attributes but also the cultivar studied. This shows that the cultivars of different genetic constitution show a differential reaction to salinity[29]. In this study, the differences between rice cultivars in germination characters might be due to the genetical factors and heredity variation among the fifteen rice cultivars under study which caused differed in germination characters.

CONCLUSIONS

The results of current study indicated that germination and seedling growth of rice cultivars were inhibited by increasing salt concentration. It was obvious that increasing levels of (NaCl) reduced germination percentage, shoot and root length, shoot fresh and dry weight, root/shoot ratio and seedling vigour index. Among fifteen tested cultivars IR-6, DR-82, DR-83 and Latify exhibited tolerance to salinity for most of the characters studied, while Sada hayat and Mashoori were the least tolerant among all.

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Table 1. Effect of water salinity levels on germination (%) and initial growth traits of rice cultivars

Water salinity levels (mM L^{-1})	Germination (%)	Shoot length (cm)	Root length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root/ shoot ratio	Seedling vigor index
0	100.00^{a}	8.28^{a}	9.79^{a}	0.85^{a}	0.0094^{a}	1.25^{a}	1807^{a}
50	79.22^{b}	5.94^{b}	6.98^{b}	0.65^{b}	0.0066^{ab}	1.25^{a}	1029^{b}
100	64.00^{c}	4.51^{c}	5.42^{c}	0.52^{c}	0.0051^{ab}	1.21^{ab}	637.3^{c}
150	58.67^{d}	2.93^{d}	3.54^{d}	0.42^{d}	0.0042^{b}	1.19^{b}	374.6^{d}
LSD (0.05) SE	1.048 2.933	0.01700 0.04756	0.06726 0.02404	0.001491 0.004171	0.001491 0.004171	0.0129 0.0373	3.75 10.5

Means followed by similar letters do not differ significantly at P=0.05.

Table 2. Effect of salinity levels on germination and initial growth traits of fifteen rice cultivars

Rice Cultivars	Germination (%)	Shoot length (cm)	Root length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root/ shoot ratio	Seedling vigor index
JJ-77	73.33^{abc}	5.21^{c}	5.952 f	0.596^{gh}	0.0062^{a}	1.30^{b}	894.5^{e}
DR-92	73.75^{abc}	5.29^{c}	6.36c	0.613^{de}	0.0064^{a}	1.20^{cd}	933.1^{cd}
Sada Hayat	69.58^{c}	5.07^{d}	6.115e	0.520^{k}	0.0053^{a}	1.21^{cd}	882.0^{e}
DR-58	71.67^{bc}	5.09^{d}	6.108e	0.503^{l}	0.0056^{a}	1.20^{cd}	879.1^{e}
IR-8	75.42^{abc}	5.28^{c}	6.365c	0.618^{d}	0.0064^{a}	1.21^{cd}	946.3^{bc}
Mashoory	71.67^{bc}	5.08^{d}	6.129 de	0.538^{j}	0.0055^{a}	1.23^{bcd}	879.4^{e}
DR-62	76.25^{abc}	5.21^{c}	6.358c	0.603^{fg}	0.0063^{a}	1.23^{bc}	948.2^{bc}
IR-6	80.00^{a}	5.89^{a}	6.718b	0.687^{b}	0.0070^{a}	1.14^{d}	1062^{a}
Shahkar	76.67^{ab}	5.29^{c}	6.329c	0.593^{h}	0.0063^{a}	1.20^{cd}	957.8^{b}
DR-61	76.25^{abc}	5.29^{c}	6.259 cd	0.595^{gh}	0.0063^{a}	1.39^{a}	920.7^{d}
Kanwal 95	75.83^{abc}	5.39^{b}	6.347c	0.606^{ef}	0.0065^{a}	1.18^{cd}	957.0^{b}
Latify	77.92^{ab}	5.95^{a}	7.032a	0.677^{c}	0.0070^{a}	1.19^{cd}	1063^{a}
DR-46	76.67^{ab}	5.28^{c}	6.332c	0.573^{i}	0.0062^{a}	1.24^{bc}	960.6^{b}
DR-82	78.75^{a}	5.95^{a}	7.020a	0.682^{bc}	0.0069^{a}	1.19^{cd}	1073^{a}
DR-83	78.33^{ab}	5.96^{a}	7.032 a	0.700^{a}	0.0071^{a}	1.29^{b}	1070^{a}
LSD (0.05) SE	2.030 5.680	0.03291 0.09210	0.04655 0.13032	0.002887 0.008078	0.002887 0.008078	0.0251 0.0723	7.262 20.33

Means followed by similar letters do not differ significantly at P=0.05.