OPEN ACCESS Plant Health 02 (01) 2023. 39-49 DOI: 10.33687/planthealth.02.01.4962

Check for updates

Available Online at EScience Press
Plant Health
ISSN: 2305-6835
https://esciencepress.net/journals/planthealth

Screening and Identification of Salt Tolerance Rice (*Oryza sativa*. L.) Genotypes at Early Seedling Stage

[a]**Arshad Ali Kaleri***, [a]**Shabana Memon**, [b]**Aisha Shereen**, [a]**Zahoor Ahmed Soomro**
a Department of Plant Breeding and Genetics, Sindh Agriculture University Tandojam, Pakistan.
b Department of Physiology, Nuclear Institute of Agriculture NIA Tandojam, Pakistan.

ARTICLE INFO

Article History
Received: April 13, 2023
Revised: June 02, 2023
Accepted: July 23, 2023

Keywords
Screening
Morpho-physiological
Seedling stage
Hoagland solution

ABSTRACT

Crop yield lose due to soil salinization is an increasing threat to agriculture worldwide. An efficient and yield based techniques for selection of salt tolerant lines has been developed under the three different levels of salinity stress such as T_1 = 00, T_2 = 6 and T_3 = 8 dS m^{-1} NaCl in green house. The current study was conducted in control condition where initially nutritional supplements were used as Hoagland solution (Hoagland solution is a hydroponic nutrient solution, which provide every essential nutrients for plant growth). The mentioned research was observed in the experimental site of Nuclear Institute of Agriculture (NIA) Tandojam to assess the response of coded as well as local cultivated lines under the salinity stress. 21 days old seedlings were transplanted at culture solution area at three different levels of sodium chloride. Twenty different lines were grown and compared with each other for morpho-physiological and yield based traits. The results exposed considerable variations among shoot length, root length, root weight, shoot weight, leaf area and chlorophyll content at different stress of NaCl. The result regarding stress observed that, genotypes GML-529, IR-72 and KANGNI-27 showed more than 50 % reduction in shoot length during stress, while genotypes IR-83, HHZ SAL-10-DT1-DT2, and SHUA-92 showed promising results for shoot length. Physiological observation revealed that HHZ SAL-10-DT1-DT2, Kharagnjia, FL-478, IR-72, SHUA-92 and GML-498 showed higher green pigment (chlorophyll content) and leaf area, however among tested lines, KSK-282, HHZ SAL-10-DT1-DT2, DR-82, IR-8, and Basmati-515 stored higher K^+ and lower Na^+ in both stress condition, which favors to our target and breeding objective.). Therefore, it is proved that above mentioned lines would be found as suitable for further breeding program regarding introducing salt tolerant rice genotypes and identified genotypes shoukd be further tested at reproductive stage for yield and yield associated traits under similar stress at different locations of saline soil of Sindh, Pakistan.

Corresponding Author: Arshad Ali Kaleri
Email: ali.breeder110@gmail.com

INTRODUCTION

Rice crop is the source of food for more than 3 billion people (Bashir *et al.,* 2020, Krishnamurthy *et al.,* 2016 and Li *et al.,* 2017). This cereal crop has a high economic value and is regarded as the second most important staple food globally. Soil salinity is a crucial issue worldwide that significantly affects plant growth in both irrigated and rain-fed regions reported by (Singh *et al.,* 2001). Osmotic stress is the initial phase of salinity stress, followed by ion toxicity that primarily affects the absorption and transportation of essential ions in plant roots (Zeng *et al.,* 2001). The extent and severity of salinity stress, differ from crop to crop, even their growth stages may also show variations which bring significant changes in physiological, biochemical, and molecular processes of plant, which ultimately resulting in a substantial decrease in yield for key agricultural crops, as highlighted by (Thitisaksakul *et al.,* 2015, Negrao *et al.,* 2017, Yuan *et al.,* 2020 and Riaz *et al.,* 202). Osmotic stress causes a reduction in the water absorption capacity of the root system and lowers the water potential of leaves. Rice crops exhibit heightened sensitivity to both biotic and abiotic stresses (Wang *et al.,* 2011). Among the abiotic factors, soil salinity is one of the significant aspects that hinders the growth and productivity of rice globally (Ruan *et al.,* 2011). Currently greater salts in the soil restricts the absorption of nutrients and water, leading to inhibited plant growth and reduced yield, that was reported by two different scientists (Ismail *et al.,* 2014) and (Arora 2019) .Consequently, the development of salt-tolerant rice varieties is very importance in coastal regions, and it remains a crucial objective in rice breeding programs, because recently it was reported that salt stress affects 20 % of global cultivable land and which is increasing continuously owing to the change in climate and anthropogenic activities (Zafar *et al*., 2015). The extent of salinity varies depending upon the plant growth stage, in most of the cultivars, more damage is observed at germination and early growth stages which indicate that salinity has negative effect on plant early growth stages (Abbas *et al.,* 2013). It also effects the germination time, root index, root and shoot length (Todka *et al.,* 2012). It is considered to be a salt-susceptible species (Kurotani *et al.,* 2015). Its salt tolerance depends on growth stage (Sahi *et al.,* 2006) and (Kanwapee *et al.,* 2011). Generally, the seedling and reproductive stages are more susceptible to salinity than the vegetative stage; roots are more sensitive than other organs (Kanwapee *et al.,* 2011). During the early vegetative and reproductive stages, rice is highly susceptible to salinity that was reported by, (Arzani *et al.,* 2016) and (Chinnusamy *et al.,* 2005). One of the primary methods for developing salt-tolerant rice cultivars through conventional breeding is to increase the genetic diversity between parental genotypes, which is typically assessed by measuring morphological and physiological differences.

MATERIALS AND METHODS

Nuclear Institute of Agriculture (NIA), Tandojam is one of the PAEC research based center, which is situated at Tandojam, Hyderabad, where current research was conducted in order to find salt tolerant genotypes under three different salt stress (control, 6 and 8 dS m^{-1} NaCl) during early seedling stage of rice crop. The design was used Randomize Complete Block Design RCBD with Factorial Design and three replications. The seedling stage of rice is considered very sensitive towards salt stress. The twenty distinct rice genotypes those were investigated during early seedling stage were : Kharaganja, RST-178, RST-177, FL-478, IR-8, IR-6, IR-72, KSK-282, HHZ-SAL-10 DT1 DT1, IR-83, GML-592, DR-83, GML-536, Shua-92, GML-498, HHZ SAL-10 DT1 DT2, Kangni-27, Basmati-515, KSK-133 and PS-2. The observation were recorded from five randomly selected plants such as shoots and roots length; shoots & roots fresh weights; shoot and root dry weight. The sample for observing the data of dry weight, fresh leaf were placed in in air drying oven for 72 hours at 80^{o}C in order to determine their dry weights in the physiological lab of Nuclear Institute of Agriculture NIA Tandojam.

RESULTS AND DISCUSSION

Salt stress is the physiological result of salt overload in plant cells, which has detrimental effects on plant metabolism. Soil is stratified as salts when the ECe (electrical conductivity of saturated soil extract) is greater than 4 dS m-1, which is roughly equivalent to 40 mM NaCl and results in an osmotic pressure of 0.2 megapascals (MPa) (Singh *et al*., 2001; USDA-ARS, 2008). Soil salts usually inhibit plant growth and reproduction through the initial phase of osmotic stress, followed by ion toxicity due to accumulation of Na+ and Cl- ions in the cytoplasm (Bhowmik *et al*., 2009). Salt stress or saline stress occurs when plants absorb excessive amounts of ions such as Na+, Ca2+, Mg2+, SO4 2-, and Cl- (Chinnusamy *et al*., 2005). Saline soils cause two main problems for plants (1) high salt concentrations reduce the water potential of the soil and (2) accumulation of high concentrations of Na+ and Cl- which are toxic to plant cells (Chinnusamy *et al*., 2005). Salt-tolerant plants have evolved several mechanisms for salt tolerance, including changes in membrane properties involved in ion uptake, translocation, compartmentalization, and salt excretion, these tolerance mechanisms promote water retention and acquisition, protect chloroplast function, and maintain ion homeostasis (Singh *et al*., 2001). Critical pathways leading to the synthesis of osmotically active metabolites (proline, glycine-betaine, sugars, etc.) and certain free radical scavenging enzymes regulate ion and water fluxes and support the removal of oxygen radicals (Bhowmik *et al*., 2009). Many researchers have now identified salt-sensitive varieties that accumulate high proline content under salt stress conditions (Theerakulpistu *et al*., 2005), and (Li *et al*., 2008).

Table 1. Mean squares of analysis variance for growth and physiological parameters under control and saline conditions in rice.

Characters	Treatments df=2	Genotypes df=19	T x G df=38	Error df=118
Growth parameters				
Shoot length (cm)	4091.62**	189.13**	105.27**	6.90
Root length (cm)	1878.42**	29.32*	23.85*	18.16
Shoot fresh weight (g)	5308.94**	519.81**	122.00**	3.42
Root fresh weight (g)	1168.83**	21.01**	19.36**	0.92
Shoot dry weight (g)	108.47**	22.23 **	3.43 **	0.70
Root dry weight (g)	120.93 **	1.98**	2.12 **	0.36
Physiological parameters				
Chlorophyll content %	50.830**	100.589**	44.250**	19.72
Leaf area (cm^2)	1258.98**	68.09**	26.45**	8.78
Na %	40.84**	0.203ns	0.17ns	0.23
K %	54.08**	0.06ns	0.070ns	0.07

Shoot Length (cm)

Considerable variations were observed among the twenty genotypes under three different salt levels. The genotype KANGNI-27 observed shorter shoot length and exposed 50 % reduction under both saline stress, however, some of the genotypes such as RST-177, FL-478, GML-538, GML-498, HHZ-SAL10-DT1-DT1 showed promising reduction in shoot length under both stress conditions (6 dS m^{-1} and 8 dS m^{-1} NaCl) respectively. Moreover, maximum shoot length was found in the genotype FL-478 and IR-83 under the both stress. The genotype KANGNI-27 was extremely affected and reflected as salt senstive genotype, under the both strain circumstances (6 and 8 dS m^{-1} NaCl). Affordably long shoot length was exhibited by a number of strains, including Kharaganja, RST-177, IR-8, IR-72, GML-529, GML-538, and GML-498 as can be seen in the (Table 2). Similar results was reported by (Shereen *et al.,* 2022) that salinity caused a significant reduction in seedling growth with varying degree of variability among these lines.

Fresh Shoot Weight (g)

It was noticed during current study that 50 % reduction was observed in HH2 SAL-10-DT1-DT2, Basmati-515, IR-72, and PS-2 under the lower salt stress (T_2), which shows that these genotypes were sensitive under the salt stress. However, maximum shoot fresh weight was observed in the genotype IR-6 followed by SHUA-92. These genotypes were highly affected by increased concentration of salt T3 = 8 ds m^{-1} NaCl, which indicate to extreme sensitive nature to salt stress. During this experiment the minimum reduction was observed in KSK-282 as shown in (Table 3). However, under salt stress greater shoot fresh weight was exhibited in the genotype IR-6 followed by SHUA-92. It has been studied that the fresh weight of all lines decreased as the level of salinity increased from 50 to 75 mM NaCl. (Shereen *et al.,* 2022) similar results were found during this experiment as given bellow.

Shoots Dry Weight (g)

Shoot dry weight was shown to vary significantly across all treatments as a result of lower and higher salt stress (T_2 &T_3). Regarding this trait, KSK-133, RST-177, and PS-2, these three genotypes showed maximum reduction of more than 50 % (82.423 %, 66.548 %, and 64.488 %, respectively), which indicate that these genotypes were incredibly susceptible to salt stress. However, greater reduction of shoot dry weight was found in RST-177 followed by SHUA-92, DR-83, HHZ SAL-10-DT1-DT1 and Basmati 515 respectively.

Table 2. Effect of different salinity (NaCl) treatments on shoot length (cm) and shoot fresh weight (cm).

	shoot length (cm)					shoot fresh weight (cm)				
	T_1= control	T_2= 6 dS m^{-1} NaCl	R.D%	T_3=8 dS m^{-1} NaCl	R.D%	T_1= control	T_2= 6 dS m^{-1} NaCl	R.D%	T_3=8 dS m^{-1} NaCl	R.D%
Kharaganja	59.44	49.66	-16.44	52.33	-11.96	18.82	16.79	-10.80	13.92	-26.03
RST-178	57.66	51.33	-10.98	51.00	-11.56	19.80	19.17	-3.14	18.25	-7.79
RST-177	68.33	51.66	-24.38	45.33	-33.65	52.90	37.82	-28.50	34.82	-34.17
FL-478	71.66	56.77	-20.77	53.66	-25.11	46.67	36.05	-22.74	30.83	-33.93
IR-8	65.33	54.33	-16.83	50.33	-22.95	28.55	22.23	-22.14	18.29	-35.95
IR-6	56.66	52.66	-7.05	50.00	-11.76	51.72	43.02	-16.82	28.42	-45.04
IR-72	63.33	55.11	-12.98	48.66	-23.15	53.77	28.92	-46.21	15.09	-71.94
KSK-282	58.33	50.77	-12.95	48.33	-17.14	28.30	28.21	-0.31	23.53	-16.84
HHZ SAL-10-DT1-DT2	62.89	59.22	-5.83	57.66	-8.30	45.12	18.23	-59.59	19.03	-57.82
IR-83	75.00	64.22	-14.36	59.66	-20.44	25.25	21.80	-13.68	17.45	-30.88
GML-529	66.11	54.66	-17.30	52.33	-20.83	26.00	18.32	-29.52	11.91	-54.19
DR-83	61.44	54.66	-11.02	57.00	-7.23	23.47	22.44	-4.40	18.16	-22.61
GML-538	72.55	56.00	-22.81	48.66	-32.92	32.34	20.76	-35.79	8.71	-73.07
SHUA-92	63.11	56.55	-10.38	53.33	-15.49	44.26	38.44	-13.15	28.90	-34.71
GML-498	71.33	50.33	-29.43	71.00	-11.50	35.90	29.00	-19.22	18.63	-48.09
HHZ 5-SAL10-DT1-DT1	64.55	52.77	-18.24	43.66	-32.35	46.32	25.55	-44.82	16.14	-65.15
KANGNI-27	90.11	52.22	-42.04	51.00	-43.40	36.70	20.00	-45.50	19.90	-45.77
BASMATI-515	73.89	51.77	-29.92	50.33	-31.88	45.29	19.89	-56.08	9.27	-79.51
PS-2	70.66	51.77	-26.73	45.66	-35.37	53.03	20.22	-61.86	13.66	-74.22
KSK-133	58.89	51.00	-13.39	31.33	-46.79	31.67	18.00	-43.17	10.24	-67.66
Average	66.567	53.878	-18.19	51.06	-22.64	37.29	25.24	-28.87	18.76	-46.27
Salinity (S) HSD 0.05%			1.13					0.80		
Genotype (G) HSD 0.05%			4.48					3.15		
S x G HSD 0.05%			8.97					6.32		

The, minimum reduction was exhibited in the genotype RST-178 and RST-177 (18 % and 16 %), respectively showing that they survived better even under the stress condition of salinity, as shown in (Table 3). Under the salt stress, greater shoot dry weight was observed in KSK-282, FL-478, IR-8, IR-6, IR-72 and HHZ SAL-10-DT1 DT1which shows that these genotypes survived better under this salinity level and considered as salt tolerant. However minimum reduction was recorded in the genotypes RST-178 and RST-177 which represent to tolerance at seedling stage. IR-72 exhibited greater shoot dry weight under this stress and accumulated lower concentration of salt. Some genotypes from followings were also used by scientist earlier for study, who reported same as highest shoot dry weight was obtained in Basmati-385 followed by KSK-133 under salt stress (Zafar *et al.,* 2015).

Root Length (cm)

Root length decreased greatly as more than 50 % in the genotype FL-478 followed by GML-498, RST-178, HHZ SAL-DT1-DT1-10, Basmati 515 and KSK-133. This showed that these genotypes were sensitive under the 6 dS m^{-1} salt stress. However maximum root length depicted in KANGNI-27 as 15.157cm. Similarly, under 8 dS m^{-1} salt stress, the genotype PS-2 showed significant decrease as 68 % and showed that it was extremely affected under this salt stress, hence it revealed to be sensitive to salt stress. However, under 8 dS m^{-1} salt stress, the genotype IR-6, DR-83 and HHZ SAL-DT1-DT1-10 showed maximum root length as 22.00 cm, 22.667cm and 20.667cm, respectively., therefor, it is concluded that some of the genotypes were sensitive at seedling stage for this character, depicting that as salt accumulates in the cells of the plant it reduces its growth and confirmed that salinity tolerance at the seedling is regulated by different set of genes (Table 4). (Zafar *et al.,* 2015). reported that under salinity stress the genotypes declined in root length Moreover, under 8 dS m^{-1} NaCl.

Table 3. Effect of different salinity (NaCl) treatments on shoot dry weight (g) and root length (cm).

	Shoot dry weight (g)					Root length (cm)				
	T_1= control	T_2= 6 dS m^{-1} NaCl	R.D%	T_3=8 dS m^{-1} NaCl	R.D%	T_1= control	T_2= 6 dS m^{-1} NaCl	R.D%	T_3= 8 dS m^{-1} NaCl	R.D%
Kharaganja	3.67	2.90	-21.13	3.01	-18.14	25.00	13.51	-45.96	16.33	-34.66
RST-178	2.90	2.76	-4.58	2.41	-16.89	25.66	8.61	-66.45	18.00	-29.87
RST-177	10.63	3.55	-66.54	2.54	-76.08	29.33	13.96	-52.38	19.33	-34.09
FL-478	4.43	4.22	-4.73	3.31	-25.33	17.66	3.37	-80.88	17.66	0.00
IR-8	5.09	4.07	-19.90	3.29	-35.36	26.66	11.57	-56.58	16.66	-37.50
IR-6	5.27	4.94	-6.32	5.22	-1.02	20.33	9.60	-52.78	22.00	8.19
IR-72	8.80	4.41	-49.88	6.10	-30.68	25.00	11.40	-54.40	17.66	-29.33
KSK-282	8.61	5.43	-36.92	4.36	-49.29	29.00	11.99	-58.65	18.33	-36.78
HH2 SAL-10-DT1-DT2	8.59	4.85	-43.45	5.53	-35.58	16.33	11.66	-28.56	18.33	12.24
IR-83	5.40	3.83	-29.01	2.71	-49.81	21.66	9.60	-55.69	13.00	-40.00
GML-529	4.23	2.41	-43.06	1.84	-56.46	18.66	4.64	-75.14	15.00	-19.64
DR-83	4.63	2.64	-42.95	1.77	-61.64	20.66	11.49	-44.40	22.66	9.67
GML-538	5.54	2.32	-58.09	2.25	-59.28	19.66	13.33	-32.20	11.49	-41.57
SHUA-92	6.23	5.35	-14.16	2.24	-64.01	23.33	13.83	-40.71	16.00	-31.42
GML-498	4.50	2.75	-38.73	1.94	-56.82	23.33	7.01	-69.95	15.33	-34.28
HHZ 5-SAL10-DT1-DT1	5.64	2.51	-55.52	2.13	-62.20	21.00	6.71	-68.04	20.66	-1.58
KANGNI-27	3.67	2.81	-23.57	1.90	-48.32	23.33	15.15	-35.04	14.66	-37.14
BASMATI-515	3.39	2.42	-28.52	1.05	-68.82	20.66	7.41	-64.14	12.66	-38.70
PS-2	1.94	0.69	-64.48	0.41	-78.89	20.00	12.20	-39.00	6.33	-68.33
KSK-133	2.60	0.45	-82.42	0.37	-85.50	26.33	10.06	-61.77	12.00	-54.43
Average	5.27	3.26	-36.76	2.72	-48.98	22.68	10.35	-54.14	16.30	-26.96
Salinity (S) HSD 0.05%			0.527					1.97		
Genotype (G) HSD 0.05%			0.77					7.79		
S x G HSD 0.05%			1.33					15.60		

Root Fresh Weight (g)

Root fresh weight also showed variable differences among most of the genotypes under both salt stresses. More than 50 % reduction was observed among the genotypes IR-8 (90 %), IR-72 (85 %), IR-83 (97 %), SHUA-92 (80 %), KANGNI-27 (92 %), Basmati-515 (83

%) and KSK-133 (87 %), respectively under the both stress as 6 and 8 dS m^{-1}NaCl. This shows that above highlighted genotypes were sensitive under both salt stresses. However, maximum root fresh weight was found in RST-177 as 6.133 g during lower saline stress of 6 dS m^{-1}. Similar to this, the genotype differences in the relative decrease for 8 dS m^{-1} salt stress were larger. However, the genotype Kharaganjia showed greater root fresh weight as 4.690 g as compared to other genotypes as shown in Table 4. However, RST-177 revealed to have maximum root fresh weight among the twenty genotypes. This showed that these genotypes were salt-sensitive towards this parameter and accumulated more salt with reduced root fresh weight. It was reported by (Rasel *et al.,* 2021) that the genotypes decreased under salt stress in all genotypes except for Basmati-385. Thus, it can be suggested that root fresh weight have negative relation with the increased concentration of salts in rice. During stress condition, the genotypes IR-6, GML-529, and Basmati-515 showed reductions of 90 %, 97 %, and 92 %, respectively, according to (Li *et al*., 2008) 95 % decrease was seen in GML-529 under treatment for 6 dS m^{-1} NaCl stress.(Shereen *et al*., 2022) also predicted the same results and found reduction in root fresh weight.

Root Dry Weight (g)

Root dry weight found variable differences among genotypes under both salt stresses (6 and 8 dS m^{-1} NaCl). Greater than 50% decrease was observed in both salt stresses for this character revealing that most of the genotypes were affected under increasing salt stresses (6 and 8 dS m^{-1} NaCl). Maximum weight of dry root was found in the genotype Basmati-515, PS-2 and KSK-133 (2.643 g, 2.223 g and 2.233 g), respectively. This shows that even stress was there but the genotypes-maintained root dry weight under 6 dS m^{-1} stress. However, other genotypes showed minimum root dry weight under this salt stress. The majority of genotypes were found to have poor root dry weight, it implies that under 8 dS m^{-1} NaCl stress, the weight of the plant decreased due to stress and salt accumulation as shown in Table 4. Root dry weight revealed significant difference under both stresses (6 and 8 dS m^{-1}). It seems that >50 % reduction was found in root dry weight and the genotypes were extremely affected under both NaCl stresses. When exposed to 6 dS m^{-1}, RST-178 showed greater root dry weight than other genotypes (3.24 g). Increased root dry weight was observed in the genotypes Basmati-515, PS-2 and KSK-133 respectively, depicting that these were tolerant for this character even after salt accumulation. Similarly, under 8 dS m^{-1} salt stress, most of the genotypes reduced considerably for root dry weight as the salt was increased (Table 4). Rice crop exhibits varying degree of sensitivities depending on crop stage, stress severity, stress duration and genotypic tolerance potential (Sakina *et al.,* 2016).

Table 4. Effect of different salinity (NaCl) treatments on root fresh weight (g) and root dry weight (g).

	Root fresh weight (g)					Root dry weight (g)				
	T_1= control	T_2= 6 dS m^{-1} NaCl	R.D%	T_3=8 dS m^{-1} NaCl	R.D%	T_1= control	T_2= 6 dS m^{-1} NaCl	R.D%	T_3=8 dS m^{-1} NaCl	R.D%
Kharaganja	13.51	4.37	-67.60	4.69	-65.28	3.23	1.243	-61.54	0.723	-77.62
RST-178	8.61	3.54	-58.85	3.85	-55.20	3.61	1.343	-62.78	0.400	-88.92
RST-177	12.93	6.13	-52.57	3.70	-71.39	4.02	1.523	-62.13	1.076	-73.23
FL-478	13.37	3.59	-73.16	2.99	-77.64	4.41	1.990	-54.87	1.076	-75.58
IR-8	12.27	2.97	-75.75	1.21	-90.14	5.06	0.390	-92.30	0.533	-89.47
IR-6	10.60	2.42	-77.14	2.75	-73.99	3.71	0.423	-88.59	0.500	-86.52
IR-72	11.40	1.76	-84.50	1.61	-85.87	4.45	0.92	-79.28	1.043	-76.58
KSK-282	11.69	2.43	-79.18	2.45	-78.98	2.31	1.92	-16.75	0.710	-69.26
HH2 SAL-10-DT1-DT2	13.10	2.16	-83.45	2.64	-79.82	1.45	1.03	-29.05	0.766	-47.34
IR-83	9.60	0.39	-95.93	0.24	-97.46	2.87	0.77	-73.01	1.123	-60.94
GML-529	4.64	2.35	-49.20	1.35	-70.75	3.84	1.40	-63.57	0.700	-81.78
DR-83	11.49	2.25	-80.35	3.11	-72.93	2.92	0.63	-78.34	0.376	-87.11
GML-538	13.13	4.19	-68.09	3.42	-73.93	2.27	0.29	-87.25	0.543	-76.12
SHUA-92	13.60	3.36	-75.24	2.71	-80.07	2.46	0.37	-84.75	1.010	-59.04

GML-498	7.01	1.21	-82.73	1.57	-77.50	2.81	0.42	-84.94	0.623	-77.81
HHZ 5-SAL10-DT1-DT1	6.37	2.00	-68.63	1.34	-78.94	5.63	0.15	-97.23	0.500	-91.12
KANGNI-27	15.19	1.69	-88.87	1.19	-92.16	3.70	0.87	-76.32	0.723	-80.45
BASMATI-515	7.04	1.10	-84.38	1.13	-83.91	4.16	2.64	-36.55	0.833	-79.99
PS-2	12.86	4.39	-65.88	3.06	-76.16	3.57	2.22	-37.83	0.700	-80.42
KSK-133	8.96	2.12	-76.32	1.13	-87.36	3.36	2.23	-33.66	0.376	-88.80
Average	10.37	3.22	-74.39	2.31	-78.47	3.39	1.24	-65.04	0.71	-77.41
Salinity (S) HSD 0.05%			0.610					0.0907		
Genotype (G) HSD 0.05%			0.8851					0.5686		
S x G HSD 0.05%			1.5330					0.9849		

Chlorophyll Content (RG)

The chlorophyll content revealed moderate effect of salt stresses at 6 and 8 dS m^{-1} NaCl stress. The genotype KSK-133 was 50 % affected (46.769 %) and can be considered as sensitive at 6 dS m^{-1} salt stress. However, most of the genotypes were not affected at this stress and were tolerant, producing more chlorophyll content. Similarly, under 8 dS m^{-1}, salt stress, the chlorophyll reduced drastically in the genotypes Basmati-515, PS-2, KSK-133 and KANGNI-27 depicted that these were sensitive under increase salt stress. However, maximum chlorophyll was found in the genotype FL-478 (46.243), SHUA-92 (45.533), GML-498 (41.077) and HHZ SAL-10-DT1-DT1 (41.967) as shown in Table .5 For both 6 and 8 dS m^{-1} NaCl salt stresses, most of the genotypes were moderately affected by both stresses. However, KSK-133 observed to have 46% affected and was sensitive under this salt stress. Though most of the genotypes conferred to be tolerant. under 8 dS m^{-1}, salt stress, the chlorophyll reduced drastically in the genotypes Basmati-515, PS-2, KSK-133 and KANGNI-27 recorded sensitivity under increase salinity stress. However maximum chlorophyll was found in the genotype FL-478, SHUA-92, GML-498 and HHZ SAL-10-DT1-DT1. These results shows that at 8 dS m^{-1} NaCl stress the weight reduced due to accumulation of increases salt and plant reduced due to stress (Table 4). These findings are with the agreements of (Li *et al*., 2008) as reported that the chlorophyll and carotenoids contents of these genotypes decreased with different intensities under salinity with least reduction in salt tolerant check (FL-478) and highest in HHZ SAL-10 DT2-DT1 & GML- 498 as reported in (Table 5).

Leaf Area (cm^2)

The mean value regarding this trait with the intensification of salt stress, such as 6 dS m^{-1} and 8 dS m^{-1} NaCl stress, leaf area decreases dramatically among genotypes. The maximum leaf area was found in the genotype IR-83 (20.893 cm^2), DR-83 (20.920 cm^2), HHZ-SAL-10-DT1-DT1 (21.593 cm^2) and KANGNI-27 (22.013 cm^2), respectively. Similarly, under 8 dS m^{-1} salt stress, the genotypes KANGNI-27 (24.223 cm^2) and FL-478 (24.993 cm^2) depicted to have more leaf area under this stress compare to other genotypes and can be considered as tolerant genotypes (Table 5). It appears that salt accumulation occurred in the leaves, but the stomata were not impacted during excess conditions of NaCl. Salinity stress drastically reduces leaf area and other growth parameters. Though it was found that as 6 and 8 dSm^{-1} stress was profound on the rice genotypes greater reduction of >50 % was depicted in most of the genotypes. RST-177, FL-478, SHUA-92, and Basmati-515 showed reduced leaf area as 51.855 %, 51. 509 %, 44.451 % and 40.711 %, respectively under 6 dSm^{-1} salt stress. Though, leaf area was found increased in the genotype IR-83, DR-83, HHZ-SAL-10-DT1-DT1 and KANGNI-27, respectively. These genotypes showed that they were tolerant even after accumulation of salt stress. Moreover, under 8 dSm^{-1} salt stress, the genotypes KANGNI-27 and FL-478 recorded to have greater leaf area under this stress compare to other genotypes and can be considered as tolerant genotypes

(Table 5). If a plant receives an enormous quantity of Na, definitely the extent of NaCl would suddenly increase up to dangerous point inside of weak and older leaves, resulting in early fallen down and a reduction in plant physiological processes. (Shereen *et al.,* 2020). These findings are similar with (Haque *et al.,* 2021).

Table 5. Effect of different salinity (NaCl) treatments on leaf area and chlorophyll content.

	Leaf area (cm^2)					Chlorophyll content (RG)				
	T_1= control	T_2= 6 dS m^{-1} NaCl	R.D%	T_3=8 dS m^{-1} NaCl	R.D%	T_1= control	T_2= 6 dS m^{-1} NaCl	R.D%& increase in fold	T_3=8 dS m^{-1} NaCl	R.D% & increase in fold
Kharaganja	26.18	18.76	-28.36	18.68	0.00	36.76	36.10	-1.81	39.55	7.57
RST-178	22.59	17.47	-22.67	21.04	-33.22	31.01	34.64	11.71	39.93	28.77
RST-177	31.72	15.27	-51.85	22.00	-4.87	35.60	41.18	15.69	41.87	17.63
FL-478	40.11	19.45	-51.50	24.99	-24.23	38.99	47.34	21.42	46.24	18.60
IR-8	27.56	19.14	-30.54	19.61	-54.86	26.74	41.36	54.66	38.26	43.07
IR-6	21.74	15.97	-26.52	21.19	-36.60	33.29	40.99	23.15	37.66	13.14
IR-72	22.76	16.45	-27.71	20.01	-2.40	37.02	36.11	-2.45	38.94	5.19
KSK-282	24.36	18.00	-26.10	21.23	-11.28	34.65	38.60	11.39	40.18	15.95
HH2 SAL-10-DT1-DT2	25.25	16.03	-36.52	15.24	-12.41	37.42	30.71	-17.93	33.34	-10.90
IR-83	34.41	20.89	-39.28	20.64	-29.09	33.17	34.76	4.79	35.38	6.64
GML-529	20.39	17.42	-14.55	18.43	-67.53	38.24	39.11	2.26	36.09	-5.63
DR-83	31.94	20.92	-34.50	16.88	-6.11	30.74	32.99	7.30	30.73	-0.03
GML-538	28.16	18.57	-34.05	19.00	-53.46	39.64	39.89	0.62	37.13	-6.33
SHUA-92	25.11	13.95	-44.45	21.58	-36.44	38.61	39.74	2.92	45.55	17.97
GML-498	20.85	18.53	-11.12	20.67	-16.90	38.20	40.01	4.73	41.07	7.52
HHZ 5-SAL10-DT1-DT1	27.43	21.59	-21.30	21.32	-0.67	34.25	39.09	14.10	41.96	22.50
KANGNI-27	32.57	22.01	-32.41	24.22	-18.77	39.63	34.04	-14.10	31.77	-19.82
BASMATI-515	25.49	15.11	-40.71	13.38	-32.74	35.10	35.83	2.08	27.21	-22.47
PS-2	19.84	17.62	-11.19	16.15	-61.05	42.13	33.22	-21.15	39.31	-6.70
KSK-133	25.72	17.64	-31.38	19.95	-14.33	36.53	19.44	-46.76	28.89	-20.92
Average	26.71	18.04	-30.8	19.81	-25.85	35.88	36.76	3.63	37.55	5.58
Salinity (S) HSD 0.05%			1.91					2.84		
Genotype (G) HSD 0.05%			2.73					4.14		
S x G HSD 0.05%			4.73					7.17		

Sodium Na^+

Na ions were accumulated more among the genotypes and had more sodium content in the rice crop genotypes. Almost all the genotyeps revealed to have more sodium % under 6 dS m^{-1} salt stress. However, under 8 dS m^{-1} salt stress, least sodium accumulation was observed in the genotypes RST-178 (1.91 %), GML-529 (1.98 %), SHUA-92 (1.86 %), GML-498 (1.98 %), HHZ-SAL-10-DTI-DT1 (1.80 %), KANGNI-27 (1.87 %), Basmati-515 (1.61 %), PS-2 (1.73 %) and KSK-133 (1.68 %), respectively. These genotypes can be considered as moderate tolerant genotypes and can be used for further selection program (Table 6). Sodium content increased under 6 dS m^{-1} than 8 dS m^{-1} salt stress. It seems that Na^+ was accumulated greater among the genotypes depicting that the rice crop sensitive due to salt stress at 6 dS m^{-1}. However, under 8 dS m^{-1} salt stress least accumulation of salt was found in the genotypes RST-178 (1.91 %), GML-529 (1.98 %), SHUA-92 (1.86 %), GML-498 (1.98%), HHZ-SAL-10-DTI-DT1 (1.80 %), KANGNI-27 (1.87 %), Basmati-515 (1.61 %), PS-2 (1.73 %) and KSK-133 (1.68 %), respectively. These genotypes can be considered as moderate tolerant genotypes and can be used for further selection program. These significant differences were observed among these rice genotypes in root to shoot partitioning of sodium (roots to shoot Na^+ at 50 mM NaCl treatment. The genotypes HHZ SAL-10 DT2-DT1 & GML-498 exhibited comparatively low ratios in their roots in comparison to FL-478, IR-6 and IR-72. Studies have indicated that accumulation of sodium in shoots depends on specific transporters proteins (high-affinity K^+ transporter: HKT) (Shereen *et al*., 2022). The results are also confined with other researchers who reported that Na^+ uptake under salt stress was obtained by various genotypes as Basmati-385, KSK-133 showing that they were sensitive.

Potassium K^+

The two treatments (6 dS m^{-1} and 8 dS m^{-1} NaCl) had significantly different potassium concentrations. Under 6 dSm^{-1} NaCl pressure, all genotypes showed less potassium buildup in the rice plant. In comparison to the normal and 6 dS m^{-1} NaCl salt strains, the genotypes obtained more potassium under 8 dS m^{-1} NaCl. At the 6 dSm^{-1} NaCl stress, GML-498 and Basmati-515 had a greater relative boom of 80 %. (74.390 and 74.665). The amounts of potassium ions were higher in the genotypes IR-8, KSK-282, HH2 SAL-10-DT1-DT2, DR-83, and Basmati-515 under 8 dSm^{-1} NaCl. Those genotypes which accumulated more potassium may be considered as tolerant genotypes. In salt sensitive lines, this reduction in growth became more pronounced with the passage of time even at the lower level of salinity (Haque *et al*., 2021). Potassium (%) was found to be variable in various genotypes under the both treatments (6 dSm^{-1} and 8 dSm^{-1} NaCl). Evidently, the genotypes with the highest potassium accumulation in the rice plant recorded levels below 6 dS m^{-1} NaCl salt strain. However, during 8 dSm^{-1} NaCl salt stress as opposed to normal and 6 dSm^{-1} NaCl salt stress, the genotypes accumulated more potassium.

Table 6. Effect of different salinity (NaCl) treatments on Na^+ and K^+ .

	Na^+ %					K^+ %				
	T_1= control	T_2= 6 dSm^{-1} NaCl	Relative increase%	T_3=8 dS m^{-1} NaCl	Relative increase%	T_1= control	T_2= 6 dSm^{-1} NaCl	Relative increase%	T_3= 8dSm^{-1} NaCl	Relative increase%
Kharaganja	1.62	3.16	94.71	2.35	44.52	2.62	0.67	74.42	1.67	36.26
RST-178	1.67	2.95	76.64	1.91	14.37	2.51	0.63	74.63	1.61	35.72
RST-177	1.50	2.65	76.64	2.10	39.72	2.45	0.71	70.92	1.66	32.20
FL-478	1.27	2.5	96.85	2.48	95.51	2.03	0.59	70.65	2.11	-3.93
IR-8	1.59	3.1	94.60	2.50	56.93	2.39	0.64	72.97	1.74	27.01
IR-6	1.51	2.08	37.94	2.13	41.25	2.24	0.53	76.11	1.76	21.66
IR-72	1.40	2.28	62.72	2.40	71.06	2.66	0.67	74.81	2.14	19.29
KSK-282	1.54	2.55	65.26	2.15	39.33	2.56	0.75	70.65	2.03	20.91
HH2 SAL-10-DT1-DT2	1.51	2.98	97.55	2.43	61.12	2.49	0.67	73.12	1.95	21.65
IR-83	1.39	2.58	85.42	2.11	51.90	2.61	0.72	72.48	1.68	35.54
GML-529	1.34	2.66	98.06	1.98	47.43	2.53	0.68	73.19	2.24	11.69
DR-83	1.75	2.06	17.85	2.54	45.06	2.48	0.57	77.07	1.80	27.61
GML-538	1.35	2.55	88.47	2.30	69.99	2.55	0.52	79.34	1.76	30.85
SHUA-92	1.28	2.51	95.79	1.86	45.44	2.41	0.44	81.60	1.72	28.63
GML-498	1.48	2.96	99.59	1.98	33.44	2.46	0.63	74.39	2.02	17.88
HHZ 5-SAL10-	1.38	2.66	92.35	1.80	29.87	2.43	0.53	78.21	1.99	18.08

DT1-DT1										
KANGNI-27	1.51	2.61	72.90	1.87	23.99	2.48	0.48	80.42	2.06	17.02
BASMATI-515	1.43	2.36	64.76	1.61	12.53	2.48	0.63	74.66	1.84	25.73
PS-2	1.53	2.97	94.11	1.73	13.26	2.78	0.67	75.95	1.70	38.87
KSK-133	1.53	2.73	77.93	1.68	9.57	2.66	0.78	70.46	1.69	36.29
Average	1.48	3.11	-112.05	2.09	-42.30	2.49	0.62	79.51	1.86	42.31
Salinity (S) HSD 0.05%			1.10					1.13		
Genotype (G) HSD 0.05%			1.15					1.14		
S x G HSD 0.05%			1.02					1.23		

CONCLUSION

It is concluded that rice genotypes possess different genetic base for salt stress condition and the ability of salt tolerance may enhance through the combination and selection of suitable genotypes at early growth stages.

CONFLICT OF INTEREST

The authors have not declared any conflict of interests.

REFERENCES

Abbas G., M. Saqib, Q. Rafique, A.U. Rahman, J. Akhtar, M.A.U. Haq and M. Nasim. 2013. Effect of salinity on grain yield and grain quality of wheat (*Triticum aestivum* L.). Pakistan Journal of Botany, 50(4): 185-189.

Arora N.K. 2019. Impact of climate change on agriculture production and its sustainable solutions. Environmental Sustainability, 2(2): 95-96.

Arzani A. and M. Ashraf. 2016. Smart engineering of genetic resources for enhanced salinity tolerance in crop plants. Critical Reviews in Plant Sciences, 35(3): 146-189.

Bashir M.A., J. Liu, Y. Geng, H. Wang, J. Pan, D. Zhang and H. Liu. 2020. Co-culture of rice and aquatic animals: An integrated system to achieve production and environmental sustainability. Journal of Cleaner Production, 249(56): 119-310.

Bhowmik S.K., S. Titov, M.M. Islam, A. Siddika, S. Sultana and M.S. Haque. 2009. Phenotypic and genotypic screening of rice genotypes at seedling stage for salt tolerance. African Journal of Biotechnology, 8(23): 291-323.

Chinnusamy V., A. Jagendorf and J.K. Zhu. 2005. Understanding and improving salt tolerance in plants. Crop Science, 45(2): 437-448.

Haque M.A., M.Y. Rafii, M.M. Yusoff, N.S. Ali, O. Yusuff, D.R. Datta and M.F. Ikbal. 2021. Advanced breeding strategies and future perspectives of salinity tolerance in rice. Agronomy, 11(8): 1631-1638.

Ismail A., S. Takeda and P. Nick. 2014. Life and death under salt stress: same players, different timing. Journal of Experimental Botany, 65(12): 2963-2979.

Kanawapee N., J. Sanitchon, P. Srihaban and P. Theerakulpisut. 2011. Genetic diversity analysis of rice cultivars (*Oryza sativa* L.) differing in salinity tolerance based on RAPD and SSR markers. Electronic Journal of Biotechnology, 14(6): 21-24.

Krishnamurthy S.L., R.K. Gautam, P.C. Sharma and D.K. Sharma. 2016. Effect of different salt stresses on agro-morphological traits and utilisation of salt stress indices for reproductive stage salt tolerance in rice. Field Crops Research, 190(5): 26-33.

Kurotani K.I., K. Hayashi, S. Hatanaka, Y. Toda, D. Ogawa, H. Ichikawa and S. Takeda. 2015. Elevated levels of CYP94 family gene expression alleviate the jasmonate response and enhance salt tolerance in rice. Plant and Cell Physiology, 56(4): 779-789.

Li N., S. Chen, X. Zhou, C. Li, J. Shao, R. Wang and A. Polle. 2008. Effect of NaCl on photosynthesis, salt accumulation and ion compartmentation in two mangrove species, Kandelia candel and Bruguiera gymnorhiza. Aquatic Botany, 88(4): 303-310.

Li Q., A. Yang and W.H. Zhang. 2017. Comparative studies on tolerance of rice genotypes differing in their tolerance to moderate salt stress. BMC Plant Biology, 17(1): 1-13.

Negrão S., S.M. Schmöckel and M. Tester. 2017. Evaluating physiological responses of plants to salinity stress. Annals of Botany, 119(1): 1-11.

Rasel M., M. Tahjib-Ul-Arif, M.A. Hossain, L. Hassan, S. Farzana and M. Brestic. 2021. Screening of salt-tolerant rice landraces by seedling stage phenotyping and dissecting biochemical determinants of tolerance mechanism. Journal of Plant Growth Regulation, 40(5): 1853-1868.

Riaz I., I. Ashraf, K.M. Ch and A. Tanveer. 2021. Training Need Assessment of rice farmers regarding weedS and their management in Punjab, Pakistan. International Journal of Agricultural Extension, 9(3): 485-492.

Ruan S. L., H.S. Ma, S.H. Wang, Y.P. Fu, Y. xin, W.Z. Liu and H.Z. Chen. 2011. Proteomic identification of OsCYP2, a rice cyclophilin that confers salt tolerance in rice (*Oryza sativa* L.) seedlings when overexpressed. BMC Plant Biology, 11(1): 1-15.

Sahi C., A. Singh, K. Kumar, E. Blumwald and A. Grover. 2006. Salt stress response in rice: genetics, molecular biology, and comparative genomics. Functional & Integrative Genomics, 6(4): 263-284.

Sakina A., I. Ahmed, A. Shahzad, M. Iqbal and M. Asif. 2016. Genetic variation for salinity tolerance in Pakistani rice (*Oryza sativa* L.) germplasm. Journal of Agronomy and Crop Science, 202(1): 25-36.

Shereen A., M.S. Asma, M. Arif, W.A.J.I.D. Mahboob and M. Khan. 2020. Salinity induced variability in morpho-physiological traits and their relationship with grain yield in rice (*Oryza sativa* L.). Pakistan Journal of Botany, 52(5): 1615-1623.

Shereen A., M.U.S. Asma, M.A. Khan, M. Ali and M. Arif. 2022. Physio-biochemical analysis of salinity tolerance in sodium contrasting rice (*Oryza sativa* L.) genotype. Pakistan Journal Botany, 54(3): 787-794.

Singh R.K., B. Mishra and V. Jetly. 2001. Segregations for alkalinity tolerance in three rice crosses. Sabrao Journal of Breeding and Genetics, 2(33): 31-34.

Theerakulpisut P., S. Bunnag and K. Kong-Ngern. 2005. Genetic diversity, salinity tolerance and physiological responses to NaCl of six rice (*Oryza sativa* L.) cultivars. Asian Journal Plant Science, 4(6): 562-573.

Thitisaksakul M., K. Tananuwong, C.F. Shoemaker, A. Chun, O.U.M. Tanadul, J.M. Labavitch and D.M. Beckles. 2015. Effects of timing and severity of salinity stress on rice (*Oryza sativa* L.) yield, grain composition, and starch functionality. Journal of Agricultural and Food Chemistry, 63(8): 2296-2304.

Todaka D., K. Nakashima, K. Shinozaki and K. Yamaguchi-Shinozaki. 2012. Toward understanding transcriptional regulatory networks in abiotic stress responses and tolerance in rice. Rice, 5(1): 1-9.

Wang X., J. Cai, D. Jiang, F. Liu, T. Dai and W. Cao. 2011. Pre-anthesis high temperature acclimation alleviates damage to the flag leaf caused by post-anthesis heat stress in wheat. Journal of Plant Physiology, 168(6): 585-593.

Yuan J., X. Wang, Y. Zhao, N. Khan, Z. Zhao, Y. Zhang, X. Wen, F. Tang, F. Wang and Z. Li. 2020. Genetic basis and identification of candidate genes for salt tolerance in rice by GWAS. Scientific Reports, 4(10): 9958.

Zafar S.A., S. Shokat, H.G.M.D. Ahmed, A. Khan, M.Z. Ali and R.M. Atif. 2015. Assessment of salinity tolerance in rice using seedling based morpho-physiological indices. Advancements in Life Sciences, 2(4): 142-149.

Zeng L., M.C. Shannon and S.M. Lesch. 2001. Timing of salinity stress affects rice growth and yield components. Agricultural Water Management, 48(3): 191-206.