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Veronica N
Regional Agricultural Research Station, ANGRAU, Andhra Pradesh, India

Venkata Ramana Rao P
Regional Agricultural Research Station, ANGRAU, Andhra Pradesh, India

Vani Praveena M
Regional Agricultural Research Station, ANGRAU, Andhra Pradesh, India

Corresponding Author:
Veronica N
Regional Agricultural Research Station, ANGRAU, Andhra Pradesh, India

Phenotyping of elite genotypes for seedling stage salinity tolerance in rice

Veronica N, Venkata Ramana Rao P and Vani Praveena M

Abstract
Salinity has become a major environmental factor that affects the growth and productivity of crops. The land under saline soils is increasing at an alarming rate. Rice, being a salt-sensitive crop is highly prone to salinity stress and the growth, yield and quality is hampered if grown under saline conditions. After drought, salinity is the second most important abiotic constraint which affects rice thereby reducing its production and productivity. The problem of salinity is more in the coastal regions in particular. Physiological phenotyping of rice genotypes for salinity tolerance will strengthen crop improvement programs to develop varieties with salinity tolerance. Hence, this study aimed at phenotyping of rice genotypes at seedling stage using hydroponics and also to examine the impact of salt stress on morphological and physiological traits. The traits shoot length, root length, shoot dry weight, root dry weight and leaf chlorophyll content reduced under salinity stress when compared to control. Besides the check FL 478, genotypes such as White Gora, Dular, CR 3818 IC 225, Rashpanjor, NPS 95, AC 43025 had a lower SES score indicating their tolerant nature. This was also reflected in terms of lesser reduction in the morphological traits such as root and shoot length as well as dry matter.

Keywords: Chlorophyll content, rice, salinity, screening, SES

Introduction
Rice (*Oryza sativa* L.) is consumed by more than half of the world's population hence is also the major staple crop in the world (Dawe *et al*., 2010) [1]. The ever increasing population is exerting a pressure on increase in the production in order to achieve the global food security. Further, nutritive quality is also important to address the malnourishment issues. Rice production is vulnerable to climate vagaries that being one of the main reasons for reduction in rice productivity. Major abiotic stress as a part of climate change include drought, salinity, submergence, high temperatures that lead to reduction in the rice yields (Jagadish *et al*., 2012) [2].

Of the abiotic stresses, salinity is a major abiotic stress that severely hampers the growth, development, and productivity of rice crop. The area under rice production is increasing under salinity and the rice productivity in coastal areas is affected mainly because by the intrusion of seawater. Rice cannot tolerate relatively high concentrations of salt hence also termed as a glycophytic plant, when exposed to salt stress it has an adverse effect on rice growth and yield which mainly is dependent on the crop stage of the occurrence of stress, the severity of stress and duration. (Zeng *et al*., 2000) [3]. The seedling stage of rice is one of the most sensitive stages of rice to salinity (Reddy *et al*., 2017) [4].

Salt stress leads to disruption of homeostasis leading to the imbalance of ions resulting in high Na^+/K^+ or low K^+/Na^+ ratios that mainly leads to leads to impairment of membrane function and structure. The cellular metabolism and cellular physiology are disturbed. On exposure to salinity rice seedlings were stunted in shoot length, root length, root number, fresh plant weight, and seedling vigor index (Ruan and Xue, 2002) [5]. It also affects the photosynthetic apparatus including the chlorophyll pigments.

The aim of the present study was to screen rice genotypes in hydroponics under saline and normal conditions at seedling stage and to identify salinity tolerant and sensitive genotypes and information from this study would help to select genotypes with high salinity tolerance for the development and utilization in breeding programmes to develop salinity tolerant rice varieties.

Material and Methods

The experiment was conducted with a set of 21 rice genotypes as listed in Table 1. They were tested for seedling stage salinity tolerance at Regional Agricultural Research Station, Maruteru. Seeds were surface sterilized using 70% ethanol followed by washing 2-3 times with distilled water. Pre-germinated seeds of each genotype was placed in a hydroponic setup in 3 replications and laid down in Completely randomized design (CRD).

Initially the rice seedlings were grown for 2-3 days in fresh water and subsequently in Hoagland's solution containing the essential macro and micro nutrients. At 3-4 leaf stage, sodium chloride was added and an EC of 6 was maintained for first 3 days. Later, the EC was increased to 12 and maintained till the end of the experiment. The pH of the solution was adjusted daily to 5.5. The nutrient solutions were changed and was replaced by fresh solution every 2-3 days by draining the old solution completely followed by rinsing three–four times with fresh solutions so as to avoid increased stress level due to sodium chloride.

Observations such as shoot length, root length, shoot dry weight, root dry weight and chlorophyll content were recorded at the end of the experiment. Shoot and roots were separated at the shoot-root junction and the length was measured and expressed in cm. The samples were oven dried for 48 h at 60ºC and the weight was recorded and expressed in g. Chlorophyll content in the leaves were recorded at the end of the experiment by 80 % acetone as per the methodology described by Porra et al. (1989) [6]. Chlorophyll a content, chlorophyll b content and the total chlorophyll content was calculated according to Lichtenthaler and Wellburn, (1983) [7]. Scoring was done according to the standard evaluation system (SES) used in rating of the visual symptoms of salt toxicity injury (Gregorio *et al*., 1997) [8].

Statistical analysis

Two-way analysis of variance (ANOVA) was performed using Statistix 8.1 package. Statistical significance of the parameter means was determined by performing Fisher's LSD test to test the statistical significance.

Results and Discussion

Shoot length

Salinity resulted in reduction of shoot and root length in all the tested genotypes. In control conditions, the shoot length ranged from 11 cm in NPS 71 to 22 cm in Black Gora. A mean reduction of 28.0% was noted under salinity conditions. Lesser reduction under salt stress in shoot length was in Rashpanjor (17.5%), Dular (19.2%), CR 3818 IC 225 (19.2%) and FL 478 (20.6%). More than 35% reduction was noted in Varshadhan and Kalakeri (Table 2).

Root length

Salinity resulted in mean reduction of 24.3% of root length. In control conditions, the root length varied from 5.9 cm (IR 20) to 9.2 cm (Apo). Under stress conditions the reduction in root length was lesser in FL 478 (10.4%) followed by Rashpanjor (13.6%), NPS 95 (14.6%) and White Gora (15.1%) and higher in CR 2862 IC 10 (30.7%), NPS 17 (31.3%) and NPS 18 (32.4%) (Table 2).

Salinity negatively affects growth and developmental parameters of rice by reducing the shoot length and root length and this was reported by Negrao *et al*., (2016), Rasel *et al*., (2020). Agnihotri *et al*., (2006) and Ali *et al*., (2014) [9, 10, 11, 12] in their experiments. The main reason being ionic toxicity and osmotic stress that results in the inhibition of plant growth reflected in terms of reduced shoot as well as root length (Singhal *et al*., 2023) [13]. These findings are in agreement with the results of this study where a reduction of both root and shoot length in all the tested genotypes was noted.

Shoot weight

Imposition of salinity resulted in reduction of both shoot and root dry weight. The mean shoot weight reduced from 1.4 g/plant to 1.0 g/plant. In control the shoot varied from 1.67 g/plant (IR 20) to 1.06 g/plant (Kalakeri). Highest shoot weight was recorded in FL 478 (1.4 g/plant) followed by CR 3818 IC 225 (1.21 g/plant), AC 43025 (1.20 g/plant), IR 20 (1.19 g/plant) and White Gora (1.17 g/plant) under stress. Lowest shoot weight was recorded in Kalakeri (0.74 g/plant) followed by NPS 18 (0.78 g/plant) and Black Gora (0.82 g/plant). Among the tested genotypes lesser reduction in shoot dry weight was observed in FL 478 (9.7 %) followed by White Gora (13.3%), AC 43025 (16.7%) and CR 3818 IC 225 (17.7%). Higher reduction was noted in IET 27051 (45.8%) (Table 3).

Root weight

The mean root weight reduced from 0.79 g/plant in control to 0.60 g/plant under salinity stress conditions. The reduction in root weight was lesser than 15% in AC 43025 (14.8%) followed by NPS 95 (14.5%), CR 3818 IC 225 (13.5%), Dular (12.1%), White Gora (10.5%) and FL 478 (9.7%). Reduction of more than 30% was recorded in IR 64-AG (33.3.%) followed by AC 43012 (33.3%), NPS 71 (34.3%), Black Gora (34.8%), IET 27865 (36.5%) and CR 2862 IC 18 (38.9%) (Table 3).

Salinity negatively affects growth and development of rice by reducing plant biomass, which results in the overall decreased growth of the plant (Agnihotri *et al.*, 2006, Ali *et al*., 2014) [11, 12]. The reduction in growth is primarily because of the excessive accumulation of Nacl leading to an imbalance in the uptake of nutrients by seedlings (Kakar *et al*., 2019) [14]. It has been reported that salinity stress up to 20 dS m^{-1} inhibited dry matter accumulation in rice (Hakim *et al*., 2010) [15]. Our results demonstrate that NaCl, treatment inhibits seedling growth in terms of reduction in shoot and root dry weight.

Chlorophyll content

Imposition of salinity led to mean reduction of chlorophyll content by 19.0% under stress. The range of chlorophyll under control conditions was 2.44 mg/g FW in Kalakeri to 1.45 mg/g FW in NPS 95 (Fig 1). Whereas under stress less than 15% reduction in chlorophyll content was evident in Rashpanjor (13.6%) followed by CR 3818 IC 225 (12.8%), AC 43025 (12.5%), Dular (11.8%), NPS 95 (11.0%), White Gora (10.3%) and FL 478 (3.8%). The reduction was more than 25% in NPS 17 (28.2%) followed by CR 2862 IC 18 (29.3%), AC 43012 (35.9%) and NPS 71 (39.4%) (Fig 2).

Exposure of plants to high salt concentration leads to high salt uptake that causes necrosis and leaf tip burn in plants (Wahome *et al*., 2001) [16]. Salt stress also leads to chlorophyll breakdown that leads to impairment of electron transport during photosynthesis. Farooq *et al*., (2021) [17] demonstrated a reduction of chlorophyll content in IR28 and Pokkali when exposed to salinity stress. In this study a mean reduction of 19.0% under salinity stress was noted. Similar reduction of chlorophyll content under stress in 12 day old rice seedlings was reported by Rahman *et al*., (2016) [18]. It has been reported that reduction in total chlorophyll content was more in susceptible genotypes when compared to tolerant genotypes (Panda *et al*., 2013) [19]. In this study the genotypes having a lower score of SES also had lesser reduction in chlorophyll content such as FL 478 (3.8%) and White Gora (10.3%).

SES under salinity stresses

SES score was recorded for all the genotypes on the 10th and 16th day after initial stress. The lowest score of 3 (Nearly normal growth, but leaf tips or few leaves whitish and rolled) classified as tolerant was observed in tolerant check FL 478 and White Gora. Score of 5 (Growth severely retarded; most leaves rolled; only a few are elongating) which are moderately tolerant was recorded in Dular, CR 3818 IC 225, Rashpanjor, NPS 95, AC 43025. Genotypes viz., CR 2862 IC 10, IR 20, Kalakeri, Apo were classified as Susceptible as there was complete cessation of growth; most leaves dry; some plants dying symptoms with a score of 7. The remaining genotypes recorded a score of 9 (Table 4).

Table 1: List of elite rice genotypes used for salinity tolerance at seedling stage

S.No	Entries
1	Varshadhan
2	Rashpanjor
3	IR 64-AG
4	NPS 17
5	NPS 18
6	NPS 71
7	NPS 95
8	FL 478
9	Black Gora
10	Kalakeri
11	White Gora
12	Dular
13	Apo
14	IR 20
15	AC 43025
16	AC 43012
17	CR 3818 IC 225
18	CR 2862 IC 10
19	CR 2862 IC 18
20	IET 27865
21	IET 27051

Table 2: Impact of salinity on shoot and root length of rice genotypes used for salinity tolerance at seedling stage

Entry	Shoot length (cm)				Root length (cm)			
	Control	Salinity	Mean	Percent reduction	Control	Salinity	Mean	Percent reduction
Varshadhan	18.2	11.3	14.8	37.9	8.5	7.1	7.8	16.5
Rashpanjor	17.1	14.1	15.6	17.5	8.1	7.0	7.6	13.6
IR 64-AG	17.2	11.3	14.3	34.3	6.5	4.8	5.7	26.2
NPS 17	16.5	11.0	13.8	33.3	6.7	4.6	5.7	31.3
NPS 18	13.2	9.3	11.3	29.5	7.4	5.0	6.2	32.4
NPS 71	11.0	7.9	9.5	28.2	7.8	5.5	6.7	29.5
NPS 95	18.2	14.3	16.3	21.4	8.2	7.0	7.6	14.6
FL 478	14.1	11.2	12.7	20.6	7.7	6.9	7.3	10.4
Black Gora	22.0	14.6	18.3	33.6	8.0	6.1	7.1	23.8
Kalakeri	21.1	13.2	17.2	37.4	8.4	6.5	7.5	22.6
White Gora	15.8	12.2	14.0	22.8	8.6	7.3	8.0	15.1
Dular	12.5	10.1	11.3	19.2	8.2	6.8	7.5	17.1
Apo	16.3	11.4	13.9	30.1	9.2	6.4	7.8	30.4
IR 20	14.3	11.0	12.7	23.1	5.9	4.2	5.1	28.8
AC 43025	15.6	12.1	13.9	22.4	6.9	5.2	6.1	24.6
AC 43012	14.2	9.3	11.8	34.5	8.2	5.8	7.0	29.3
CR 3818 IC 225	15.1	12.2	13.7	19.2	7.6	5.9	6.8	22.4
CR 2862 IC 10	16.2	11.2	13.7	30.9	7.5	5.2	6.4	30.7
CR 2862 IC 18	12.4	9.4	10.9	24.2	6.9	4.9	5.9	29.0
IET 27865	15.1	11.2	13.2	25.8	9.0	6.5	7.8	27.8
IET 27051	13.4	9.0	11.2	32.8	8.1	6.0	7.1	25.9
Mean	15.7	11.3		28.0	7.8	5.9		24.3
LSD (T)	1.38				0.21			
LSD (V)	0.86				0.42			
LSD (TxV)	1.58				0.89			
CV (%)	8.5				7.4			

Table 3: Impact of salinity on shoot and root weight (g/plant) of rice genotypes used for salinity tolerance at seedling stage

Entry	Shoot weight (g/plant)				Root weight (g/plant)			
	Control	Salinity	Mean	Percent reduction	Control	Salinity	Mean	Percent reduction
Varshadhan	1.58	0.95	1.27	39.9	0.89	0.65	0.77	27.0
Rashpanjor	1.42	1.09	1.26	23.2	0.64	0.54	0.59	15.6
IR 64-AG	1.32	0.95	1.14	28.0	0.84	0.56	0.70	33.3
NPS 17	1.66	1.05	1.36	36.7	0.69	0.55	0.62	20.3
NPS 18	1.14	0.78	0.96	31.6	0.74	0.56	0.65	24.3
NPS 71	1.33	0.98	1.16	26.3	0.70	0.46	0.58	34.3
NPS 95	1.11	0.84	0.98	24.3	0.69	0.59	0.64	14.5
FL 478	1.55	1.40	1.48	9.7	0.62	0.56	0.59	9.7
Black Gora	1.22	0.82	1.02	32.8	0.89	0.58	0.74	34.8
Kalakeri	1.06	0.74	0.90	30.2	0.81	0.63	0.72	22.2
White Gora	1.35	1.17	1.26	13.3	0.76	0.68	0.72	10.5
Dular	1.27	1.01	1.14	20.5	0.66	0.58	0.62	12.1
Apo	1.28	0.90	1.09	29.7	0.78	0.61	0.70	21.8
IR 20	1.67	1.19	1.43	28.7	0.81	0.64	0.73	21.0
AC 43025	1.44	1.20	1.32	16.7	0.88	0.75	0.82	14.8
AC 43012	1.52	0.85	1.19	44.1	0.96	0.64	0.80	33.3
CR 3818 IC 225	1.47	1.21	1.34	17.7	0.74	0.64	0.69	13.5
CR 2862 IC 10	1.66	1.15	1.41	30.7	0.88	0.71	0.80	19.3
CR 2862 IC 18	1.55	1.04	1.30	32.9	0.95	0.58	0.77	38.9
IET 27865	1.47	1.01	1.24	31.3	0.85	0.54	0.70	36.5
IET 27051	1.55	0.84	1.20	45.8	0.80	0.58	0.69	27.5
Mean	1.41	1.01			0.79	0.60		
LSD (T)	0.058				0.010			
LSD (V)	0.089				0.026			
LSD (TxV)	0.154				0.056			
CV (%)	6.58				8.44			

Table 4: Modified standard evaluation score (SES) for salinity stress at seedling stage

Score	Observation	Tolerance	Genotypes
1	Normal growth, no leaf symptoms	Highly tolerant	FL 478
3	Nearly normal growth, but leaf tips or few leaves whitish and rolled	Tolerant	White Gora
5	Growth severely retarded; most leaves rolled; only a few are elongating	Moderately tolerant	Dular, CR 3818 IC 225, Rashpanjor, NPS 95, AC 43025
7	Complete cessation of growth; most leaves dry; some plants dying	Susceptible	CR 2862 IC 10, IR 20, Kalakeri, Apo
9	Almost all plants dead or dying	Highly Susceptible	IET 27865, IR 64-AG, CR 2862 IC 18, NPS 71, NPS 17, NPS 18, AC 43012, Varshadhan, Black Gora, IET 27051

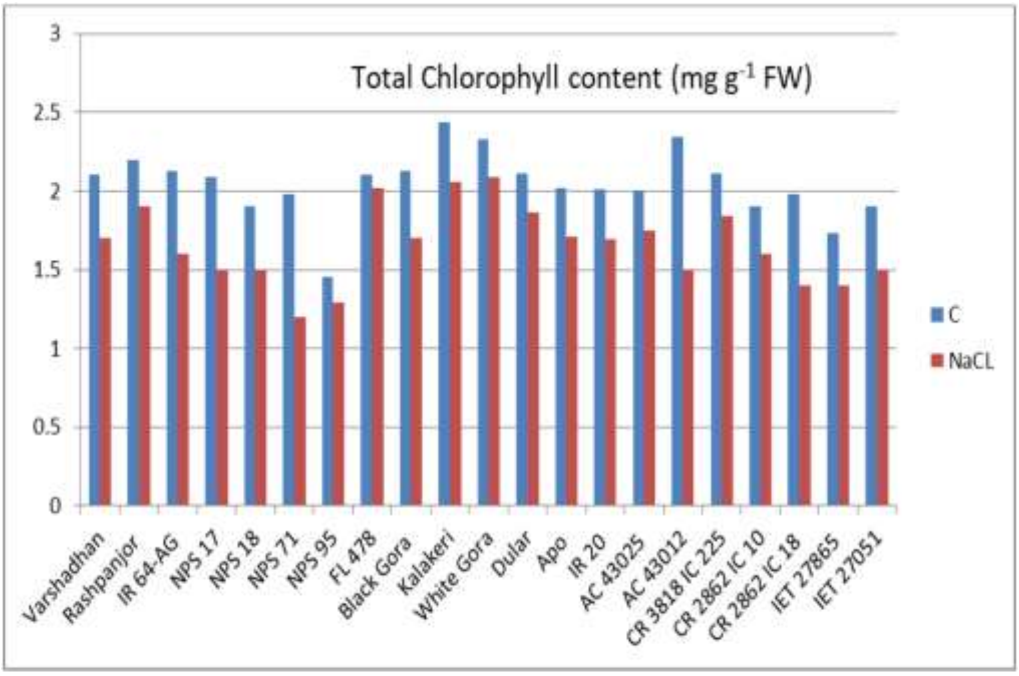

Fig 1: Impact of salinity stress on total chlorophyll content (mg/g FW) of rice genotypes.

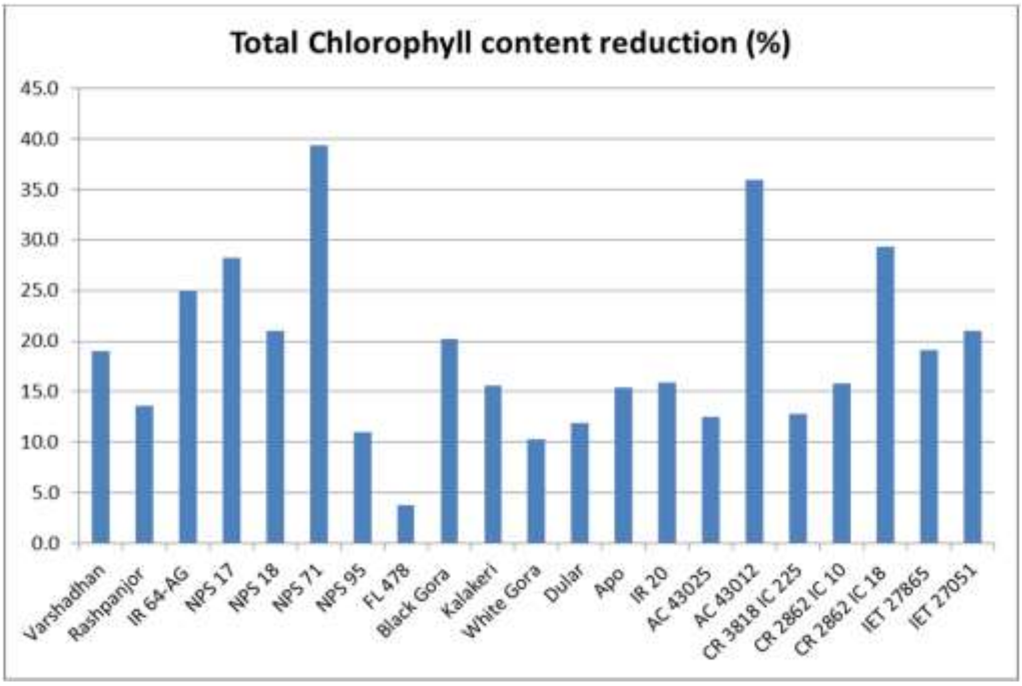

Fig 2: Impact of salinity stress on reduction of total chlorophyll content (mg/g FW) of rice genotypes

Conclusion

Salinity is a major abiotic stress that affects morpho-physiological processes in a plant. Under salinity stress, root and shoot length as well as dry matter reduced coupled with a reduction in chlorophyll content. Besides the check FL 478, genotypes such as White Gora, Dular, CR 3818 IC 225, Rashpanjor, NPS 95, AC 43025 had a lower SES score indicating their tolerant nature. They can be identified as salinity tolerant genotypes and they can used as donors in further breeding programmes.

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