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Screening of rice (*Oryza sativa* L.) genotypes for salinity tolerance under laboratory condition

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Abstract
The present experiment was conducted in order to screen 12 rice genotypes including two checks to ascertain the salinity tolerance capacity of each genotype under five salt (NaCl) concentrations in the laboratory of Department of Seed Science and Technology, University of Agricultural Sciences, Raichur during the year 2018. The experiment consisted of five levels of salinity (0, 40, 60, 80 and 100 mM NaCl) and twelve different rice genotypes. The results revealed that among the five levels of salinity S_1: 0 mM NaCl recorded significantly higher seed germination (90.0 %) and vigour (3011) compared to higher salinity levels and among the various genotypes GNV 1109 with 94.1 per cent seed germination and seedling vigour (3533) was found to be salinity tolerant. However, irrespective of the genotypes, the seed germination and seedling vigour went on decreasing with increase in salinity levels.

Keywords: Rice, salinity levels, genotypes, seed germination, seedling vigour

Introduction
Rice (*Oryza sativa*) is a major monocotyledonous cereal that plays an important role in national food security as it is a staple food for more than half of the world's population. It belongs to the genus *Oryza* and has two cultivated and 22 wild species. The cultivated species are *Oryza sativa* and *Oryza glaberrima*. About 90 per cent of the world's rice is produced and consumed in Asia and India ranks second in rice production next to China. Thailand, Vietnam, China and the United States are the world's largest exporters. In India, rice is grown in an area of 42.9 million hectare with a production of 111.1 million tonnes and an average productivity of 2.58 tonnes per hectare (Anon., 2018) [5]. In Karnataka, rice is grown in an area of 1.34 million hectare with an annual production of 3.52 milion tonnes and productivity of 2649 kg per ha (Anon., 2017) [2].

In India rice is grown under wide varying conditions of altitude and climate and is mainly grown in two types of soils *i.e.*, uplands and low lands. However, the abiotic and biotic stresses can reduce rice yield. Among them, salt-sensitivity being the major one. Salinity can develop naturally or by human intervention. Out of 230 million ha of the world's irrigated land, 45 million ha (20%) is being salt-affected (Hoang *et al*., 2016) [14]. Ion toxification, nutritional disorders and poor soil physical conditions in salt-affected soils will reduce the productivity of crop.

Rice is reported to be relatively tolerant to salinity stress during germination, active tillering and towards maturity, but sensitive during early seedling and reproductive stages. An addition of as little as 50 mM NaCl in the soil can reduce rice yield significantly. Generally, salinity causes two types of stresses on plants: osmotic and ionic. Osmotic stress occurs after the concentration of salts around the roots of the plant increase beyond a threshold tolerance level (3 d Sm^{-1}) while ionic stress occurs when the concentration of salt in old leaves reaches a toxic level due to the influx of large amounts of Na^+ into the plant, resulting in increased Na^+ concentrations in the vacuole and cytoplasm leading to interruption of metabolic processes and cell death (Hoang *et al*., 2016) [14].

Efficient strategies are required for effective utilization of saline lands for crop production. Improvement of salinity tolerance in crop species is one of the potential strategy in overcoming salinity problems in agriculture (Flowers, 2004; Yamaguchi and Blumwald, 2005) [9, 39]. Development of salt tolerant genotypes through conventional breeding is very slow due to the complexity of salt tolerance and lack of reliable traits for selection (Yamaguchi

and Blumwald, 2005) [39]. Screening is an essential part of plant breeding and several screening and selection programs have been proposed for improvement of salt tolerance (Dewey, 1962; Kingsbury & Epstein, 1984 and Gorham, 1997) [8, 24, 10]. Salinity tolerance screening based on growth, yield and its attributes has become the method of choice by labs worldwide. Recently seed physiological parameters have also gained recognition as an important criteria for screening salinity tolerance in plants at early seedling stage due to the reliability of information attained. Therefore, the present study was undertaken to screen twelve rice genotypes for salinity tolerance.

Material and Methods

The seed material for the present experiment was collected from the Senior Rice Breeder, Agricultural Research Station, Gangavathi. University of Agricultural Sciences, Raichur. The different salinity levels (0, 40, 60, 80, 100 mM NaCl) were created by preparing the required NaCl salt concentrations and the germination papers were soaked in the respective salt solution for 30 minutes and the germination test was conducted to screen 10 rice genotypes (G_1: IET 22066, G_2: IET 24767, G_3: GNV 1108, G_4: GNV 16-02, G_5: RNV 15048, G_6: IET 25497, G_7: IET 26241, G_8: GNV 1109, G_9: GNV10-89, G_{10}: MTU-1010) along with two checks, BPT 5204 (saline sensitive check) and CSR 22 (saline tolerant check) based on seed germination and seedling vigour in the laboratory of Department of Seed Science and Technology, College of Agriculture, UAS Raichur. The experiment consisted of 60 treatments laid out in 2 Factorial CRD in four replications with five salt concentrations as factor I and twelve genotypes as factor II. The experimental data was analyzed as per Sundarrajan *et al.* (1972).

The below mentioned observations were recorded

Seed germination (%)

The standard germination test was carried out by following between Paper Method (ISTA, 2013). Four replicates of hundred seeds were taken from each treatment and were germinated in germination chamber maintained at 25 ± 2 ^{0}C temperature and 90±5 percent relative humidity. Then the first count was taken on 5th day and the final count on 14th day. The numbers of normal seedlings from each replication were counted and the mean germination was expressed in percentage.

Seedling vigour parameters

At the time of germination count in roll towel method, ten normal seedlings were selected randomly from each replication and root and shoot lengths were measured and values were expressed in centimeter. The seedlings were later placed in a butter paper cover and dried shade for 24 h and then kept in a hot air oven maintained at 70 ± 2 ^{0}C for 24 hours. Dry weight was recorded and the mean values were expressed in milligram. The seedling vigour index I was computed using the formula as suggested by Abdul-Baki and Anderson (1973).

Results and Discussion

The seed germination (%) was significantly influenced by the salinity levels, genotypes and their interactions (Table 1). Among the salinity levels, significantly higher seed germination (90.0%) was recorded by S_1 (0 mM NaCl) compared to all other salinity levels. However, with the increase in salinity, the seed germination percentage went on decreasing and the lowest seed germination (81.0 %) was registered in S_5 (100 mM NaCl), however S_1 (0 mM NaCl) was on par with S_2 (40 mM NaCl) which recorded 89.0 per cent seed germination. Germination is a critical part of plant life cycle. The ability of the seeds to germinate at high salinity in the soil is therefore of crucial importance for the survival and perpetuation of the plants. Salinity is one of the environmental factors having a critical influence on seed germination, seed physiology and plant establishment (Hashemi and Armaki, 2015) [12]. The reduction of germination at high salt concentration might be mainly due to osmotic stress (Heenan *et al*., 1988) [13]. The plants exposed to high concentrations of NaCl reduce imbibition of water due to osmotic potential of the medium (Safarnezhad and Hamidi, 2008) [32] which negatively affects seed germination (Jamil *et al*., 2006; Munns and Tester, 2008) [18, 26].

Among the genotypes, GNV 1109 (G_8) has recorded significantly higher seed germination (94.1%), while the lowest (74.9%) was recorded by GNV 1108 (G_3), however GNV 1109 (G_8) was on par with IET 25497 (G_6) which recorded 93.7 per cent seed germination. The final germination of all the genotypes decreased as the salinity level increased. Higher salinity during seed germination often causes osmotic and/or specific toxicity which may reduce or retard seed germination (Waisel, 1972; Basalah, 1991) [38, 7]. Some of the genotypes were tolerant against the complications of salt stress (Jeannette *et al*., 2002) [20]. It was noticed that sodium chloride reduces the α-amylase activity in germinating seeds in some rice genotypes even at low NaCl concentrations Shereen *et al.* (2011) [35] with tolerant lines exhibited higher enzymatic activity than the sensitive ones. Results of the present study showed that the response to salinity stress during germination was variable among the genotypes. This is because the sensitivity of plants to salinity depends on plant species and their developmental stage Prado *et al*. (2000) [30].

The interaction effect also showed a significant variation for germination (60.0% to 96.0%). Soil salinity can significantly inhibit seed germination and seedling growth, due to the combined effects of high osmotic potential and specific ion toxicity. Reduction in seed germination of wheat genotypes under NaCl treatments have been reported earlier (Khan and Panda, 2005; Khan *et al*., 2006; Saboora *et al*., 2006; Akbari *et al*., 2007) [23, 22, 2].

Similar to seed germination, the shoot and root length also varied significantly at different salinity levels wherein the highest shoot and root length (14.7 and 18.6 cm) was recorded at 0 mM NaCl (S_1) compared to other salinity levels and we noticed a inverse relation between seedling length and salinity levels, where in the lowest shoot and root length (12.7 and 16.7 cm) was noticed at higher salinity level, 100 mM NaCl (S_5). This was mainly due to toxic effects of NaCl and unbalanced nutrient uptake by the seedlings Ali *et al.* (2014) [3] and also due to slow down of water uptake by the plant (Hussain and Rehman, 1997 and Jeannette *et al*., 2002) [15, 20]. Salinity can rapidly inhibit the root growth Neumann (1993) [29] and its capacity for water and essential mineral nutrition uptake from soil (Hakim *et al*., 2010) [11]. Under salinity stress, high amount of ROS generated in root cells might have damaged the existing active cells, which had a negative effect on cell proliferation and also negatively affects cell wall reconstruction and re-synthesis, which would interfere with roots cell expansion and division (Long *et al*., 2015) [25].

Among the genotypes, the shoot and root length varied significantly, wherein significantly higher shoot and root

length (17.5 and 20.0 cm) was obtained in GNV 1109 (G_8), which was on par with IET 25497 (G_6) and MTU 1010 (G_{10}), which recorded 17.2 cm and 16.5 cm shoot length and 19.2 and 19.1 cm root length, respectively. Whereas, the lowest shoot and root length (10.1 and 14.2 cm) was recorded in BPT 5204 (G_{12}) and GNV 1108 (G_3), respectively. It was noticed that the germination, seedling growth and development was better in the tolerant genotype than the sensitive ones (Safitri *et al*., 2017) [33]. This study indicates that reduction in seedling growth is a good indicator for sensitivity of rice genotypes to salt stress (Islam and Karim, 2011) [17]. Reduction of seedling length is a common phenomenon in many crop plants grown under saline conditions (Javed and Khan, 1995; Karim *et al*., 1992) [19, 21]. Here tolerant genotype may be having higher capacity to withstand an inhibitory effect of NaCl compared to sensitive one and were found to be a stress tolerant by attaining more root length compared to other genotypes. From this it can be concluded that, while, screening rice genotypes for salinity tolerance, its shoot and root may be considered as selection criteria (Safitri *et al*., 2017) [33]. The interaction due to salinity levels and genotypes was found to be non significant for shoot and root length.

Similarly the seedling dry weight (mg) and seedling vigour index (SVI) was also significantly affected by both the salinity levels and genotypes. The seedling dry weight and seedling vigour index went on decreasing with the increase in salinity levels irrespective of the genotypes (Segatoleslami, 2010) [34]. Where in, the higher salinity level, S_5 (100 mM NaCl), recorded the lowest seedling dry weight and SVI (120.5 mg 2409). Accumulation of salt in the seedlings may affect the translocation of food and water to the developing seedling which may not grow properly under saline condition (Munns, 2002) [27] and this reduction in growth ultimately leads to lesser dry weight of the seedlings. Another cause of growth inhibition under NaCl stress could be imbalanced uptake of mineral nutrients due to competition with Na^+ (Ashraf & Sarwar, 2002) [6]. Since seed vigour index is obtained by multiplying seedling length and germination, reduction in seed vigor was expected.

The seedlings of rice genotypes used in the present study varied significantly for seedling dry weight and SVI, where in significantly higher seedling dry weight and SVI (190.9 mg and 3533) and the least (92.7 mg and 1993) were recorded by GNV 1109 (G_8) and GNV 1108 (G_3), respectively. Reduction in dry weight of the genotypes was also attributed by decreased water potential of rooting medium and growth inhibition related to osmotic effects under salt stress (Munns *et al*., 1995) [28] and our results are in confirmatory with the findings of Shereen *et al*. (2005) [36] who observed a significant reduction in seedling growth under salinity. As mentioned above with increasing salinity the shoot and root length decreased among the genotypes, which ultimately lead to variation in vigour index of the genotypes used in the present study. The interaction effect showed a non significant variation for seedling dry weight and SVI.

Table 1: Influence of NaCl salinity on seed germination, shoot length, root length, seedling dry weight and seedling vigour index of rice genotypes

Treatment	Germination (%)	Shoot length (cm)	Root length (cm)	Seedling dry weight (mg)	Seedling vigour index
Salinity level (S)					
S_1 : 0	90.0	14.7	18.6	134.8	3011
S_2 : 40 mM	89.0	14.4	18.2	130.9	2917
S_3 : 60 mM	87.5	13.8	17.8	128.0	2785
S_4 : 80 mM	84.4	13.3	17.3	124.7	2599
S_5 : 100 mM	81.0	12.7	16.7	120.5	2409
Mean	86.4	13.8	17.7	127.8	2744
S. Em ±	0.5	0.3	0.2	2.1	35
CD @ 1%	1.4	0.7	0.6	5.8	97
Genotype (G)					
G_1: IET 22066	80.9	12.0	16.0	103.7	2270
G_2: IET 24767	87.0	13.1	18.3	116.9	2744
G_3: GNV 1108	74.9	12.2	14.2	92.7	1993
G_4: GNV 1602	90.8	13.4	18.7	124.9	2914
G_5: RNV 15048	81.5	11.9	16.5	104.8	2323
G_6: IET 25497	93.7	17.2	19.2	180.6	3408
G_7: IET 26241	86.7	12.6	18.0	112.8	2654
G_8: GNV 1109	94.1	17.5	20.0	190.9	3533
G_9: GNV 1089	87.4	12.8	17.8	109.9	2679
G_{10}: MTU 1010	91.6	16.5	19.1	167.1	3261
G_{11}: CSR 22	85.4	15.9	18.2	130.4	2920
G_{12}: BPT 5204	82.6	10.1	16.7	99.0	2228
Mean	86.4	13.8	17.7	127.8	2744
S. Em ±	0.8	0.4	0.4	3.2	54
CD @ 1 %	2.2	1.1	1.0	8.9	150
Interaction (SXG)					
S_1G_1	85.8	13.0	16.8	108.0	2544
S_1G_2	90.5	14.2	19.2	125.0	3031
S_1G_3	84.0	13.2	15.0	99.8	2359
S_1G_4	92.0	14.5	19.6	128.8	3136
S_1G_5	86.5	12.7	17.3	108.5	2596
S_1G_6	95.5	18.0	20.1	187.5	3630
S_1G_7	90.0	13.5	19.0	115.8	2917
S_1G_8	96.0	18.3	20.9	209.5	3762
S_1G_9	90.8	13.8	18.8	114.0	2959

S_1G_{10}	94.5	17.3	20.0	173.5	3524
S_1G_{11}	88.0	16.7	19.1	143.3	3150
S_1G_{12}	87.0	11.4	17.6	104.5	2528
S_2G_1	85.5	12.7	16.5	106.0	2486
S_2G_2	89.3	13.9	18.7	120.5	2904
S_2G_3	81.0	12.9	14.7	94.8	2240
S_2G_4	91.8	14.1	19.2	126.3	3057
S_2G_5	86.0	12.5	17.0	106.5	2528
S_2G_6	94.5	17.8	19.8	183.8	3558
S_2G_7	89.0	13.2	18.5	115.0	2820
S_2G_8	94.8	18.0	20.5	198.5	3653
S_2G_9	89.5	13.4	18.4	113.3	2845
S_2G_{10}	92.5	17.0	19.7	170.3	3399
S_2G_{11}	87.5	16.5	18.6	134.3	3079
S_2G_{12}	86.5	10.8	17.3	102.3	2432
S_3G_1	82.8	12.2	16.1	103.3	2338
S_3G_2	88.0	13.2	18.4	115.0	2779
S_3G_3	80.5	12.3	14.3	93.5	2139
S_3G_4	90.8	13.5	18.7	125.5	2913
S_3G_5	83.0	12.0	16.7	103.8	2381
S_3G_6	94.0	17.3	19.3	183.0	3426
S_3G_7	87.5	12.6	18.1	114.0	2680
S_3G_8	94.3	17.6	20.1	193.3	3552
S_3G_9	88.3	12.8	17.9	110.0	2703
S_3G_{10}	91.0	16.5	19.1	166.5	3251
S_3G_{11}	87.0	16.0	18.3	128.5	2981
S_3G_{12}	83.5	10.4	16.9	99.3	2277
S_4G_1	77.5	11.4	15.6	102.0	2088
S_4G_2	85.0	12.7	17.9	112.0	2607
S_4G_3	69.0	11.7	13.9	91.5	1761
S_4G_4	90.5	12.8	18.3	123.8	2814
S_4G_5	78.0	11.6	16.2	103.0	2162
S_4G_6	93.5	16.7	18.7	180.3	3305
S_4G_7	84.8	12.1	17.6	111.0	2508
S_4G_8	94.0	17.0	19.6	180.8	3442
S_4G_9	85.3	12.3	17.3	107.0	2513
S_4G_{10}	90.8	16.0	18.5	165.3	3132
S_4G_{11}	84.0	15.6	17.8	124.8	2809
S_4G_{12}	80.0	9.2	16.3	95.3	2043
S_5G_1	73.0	11.0	15.0	99.0	1894
S_5G_2	82.3	11.8	17.4	111.8	2400
S_5G_3	60.0	11.1	13.3	84.0	1468
S_5G_4	89.0	12.0	17.8	120.0	2651
S_5G_5	74.0	10.9	15.4	102.3	1947
S_5G_6	91.0	16.1	18.2	168.3	3120
S_5G_7	82.0	11.6	17.0	108.0	2346
S_5G_8	91.5	16.5	19.0	172.3	3255
S_5G_9	83.3	11.7	16.9	105.3	2375
S_5G_{10}	89.3	15.6	18.1	160.0	3001
S_5G_{11}	80.5	15.0	17.1	121.3	2583
S_5G_{12}	76.0	8.8	15.6	93.5	1863
Mean	86.4	13.8	17.7	127.8	2744
S. Em ±	1.7	0.9	0.8	7.1	120
CD @ 1 %	4.9	NS	NS	NS	NS

Conclusion

From the results of the study it was concluded that with increase in salinity level there was a decrease in germination (%), seedling vigour and other seed quality parameters in all the genotypes and among the genotypes, IET 24767, GNV-1602, IET 25497, IET 26241, GNV 1109, GNV 1089, MTU 1010 registered higher seed germination than the tolerant check (CSR 22) and they can be categorized as salinity tolerant genotypes. So, we can conclude that screening at early seedling stage is best to categorize the genotypes into tolerant and sensitive ones to salinity.

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