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Evaluation of rice genotypes for salinity tolerance at seedling stage

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Abstract
The present investigation consists of thirty rice genotypes and the experiment was conducted during *Kharif*-2016 in laboratory condition in the department of PMB & GE. Salinity screening for thirty rice genotypes was performed at the seedling stages in the hydroponic system. Phenotyping of the germplasm was done at EC 6dS/m at seedling stage. Based on modified standard evaluation score for visual salt injury at seedling stage, three (IR-68144-2B-2-2-3-166, IR-68144-2B-2-2-3-1-120 and IR-68144-2B-2-2-3-1-127) genotypes were salt tolerant, ten (IR-91167-133-1-1-2-3, RP-4993-55-14-3-5-1, IR-91167-31-3-1-33, IR-92953-49-1-3, IR-84722-82-2-3-3-3, IR-91167-99-1-1-1-3, IR-91171-66-3-2-1-3, R-RHZ-14-7, IR-82475-110-2-2-1-2 and IR-92966-95-1-3) were moderately tolerant, nine(IR-64, IR-91175-27-1-3-1-3, RP-BIO-5478-185M, IR-92960-75-1-3, NUD-3, IR-91171-70-3-3, Ayaar, IR-91158-85-3-2-33 and IR-836 68-35-2-2-2) were susceptible and eight (NDR-359, MTU-1010, Taramon, IR-83294-66-2-2-3-2, R-RHZSM-4, R-RHZ-2, Sambha Mansuri and Swarna genotypes were highly susceptible. The indentified salt tolerant genotypes can be potential germplam sources for future breeding program.

Keywords: Rice, Salinity, Hydrophonics, Seedling stage, Rice

Introduction
Rice is the seed of the grass species *Oryza sativa* L. (Asian rice) or *Oryza glaberrima* (African rice). As a cereal grain, it is the most widely consumed staple food for a large part of the world's human population, especially in Asia. It is the agricultural commodity with the third-highest worldwide production (rice, 741.5 million tonnes in 2014), after sugarcane (1.9 billion tonnes) and maize (1.0 billion tonnes). India is the world's second largest producer of rice, wheat and other cereals. The huge demand for cereals in the global market is creating an excellent environment for the export of Indian cereal products. According to the final estimate for the year 2014-15 by Ministry of Agriculture of India, the production of rice stood at 105.48 million tonnes. (According to APDEA report, 2016).

The high food quantities of rice grain at one hand, and the intensive growth of population in the developing countries on the other, have predetermined the wide cultivation of this crop practically in all situations including saline-alkali soils. However, considering the current rate of population growth at the global level and in our country, the requirement of rice by 2020 AD is estimated to be around 800 and 140 million tonnes, respectively. This increased productivity of rice therefore, must come from less land, water, labour and other inputs. This reducing trend of agricultural land availability would be overcome with utilization of an area estimated at more than 22 m ha in Asia alone. Both saline and alkali soils are wide spread in inland areas as that in U.P. Salinity as an abiotic stress widely limit the crop production severely (Shannon, 1998) [11]. The pH of saline soils generally ranges from 7- 8.5 (Mengel *et al.* 2001) [8]. The arid and semi-arid zones, characterized by low precipitation and high evaporation are the most affected due to minimum lixiviation of salt from the soil profile resulting in increased salt accumulation. Salinity prone areas found in the arid and semiarid zones are usually accounted to the accumulation of salts over ages**.** The effect of salinity on rice is many fold, leading to inhibition of germination, difficulties in crop area establishment, leaf area development, decrease in dry matter production, delay in seed set and also even

sterility can occur (Khatun *et al*, 1995; Asch *et al.*, 2001) [7, 3]. It has been well documented that the effect of salinity on seedling growth, seedling establishment, grain yield components such as spikelet number, tiller number has successively lead to a reduction in grain yield (Khatun *et al.*, 1995; and Zeng *et al.*, 2003) [6, 13].

Materials and Methods

Plant materials:

A total of 30 traditional and improved rice genotypes were used in the study *viz*., IR91167-31-3-1-33, IR91167-99-1-1-1-3, IR91167-133-1-1-2-3, IR91171-66-3-2-1-3, IR91175-27-1-3-1-3, IR91158-85-3-2-3-3, IR92953-49-1-3, IR92960-75-1-3, IR92966-95-1-3, IR92971-70-3-3, IR68144-2B-2-2-3-1-166, IR82475-110-2-2-1-2, IR83294-66-2-2-3-2, IR83668-35-2-2-2, IR84722-82-2-3-3-3, RP-BIO-5478-185M, RP4993-55-14-3-5-1, R-RHZ-2, R-RHZSM-4, R-RHZIH-7, IR68144-2B-2-2-3-1-120, IR68144-2B-2-2-3-1-127, Taramon, Swarna, IR-64, NUD-3, NDR-359, Ayaar, Sambha Mansuri and MTU-1010.

Screening of rice genotypes at the seedling stage

The genotypes were evaluated for their tolerance to salinity under laboratery of PMB and GE department of Narendra Deva University of Agriculture and Technology Kumarganj, Faizabad using standard protocol (Gregorio *et al.*, 1997). Rice is very sensitive to salinity at seedling stage. Its height, root length, emergence of new roots, and dry matter decrease significantly at EC (electrical conductivity) 5-6 **dS**/m" (Pearson et al., 1966, Akbar and Yabuno 1974) [10, 1]. Salinity stress at early seedling stage manifest on the first leaf, followed by the second, and finally on the growing leaf. Salinity suppresses leaf elongation and formation of new leaves. Photosynthetic function and chlorophyll content were inversely proportional to salinity level (Ota and Yasue 1962). The screening technique developed is based on the ability of seedlings to grow in salinized nutrient solution.

Materials and instruments required

To conduct the screening at seedling stage, need some basic equipments and materials required are given below:

- pH meter
- EC meter
- Balance (1000 g capacity and 0.0001 g readability)
- NaOH and HCl
- NaCl (analytical grade)
- Reagents (analytical grade) for nutrient solution
- Germinator oven
- Volumetric flasks: 100 and 200 ml capacity
- Graduated cylinders: 25, 50, and 100 ml
- Plastic trays: 12 liter capacity (Dark color trays are preferred)
- Beaker: 1000 ml
- Thermacole (50 and 2 cm thick for making seedling floats)
- Mixing containers: Cylindrical plastic containers, 50 liter and 100 liter capacity.

Preparation of stock solutions

Proper preparation of stock solutions was done essential to avoid nutrient deficiencies and mineral toxicities, not attributed to salinity stress. Fresh stock solutions were prepared every two months. The required amounts of each element for a two-month period, for preparation of 4 liter stock solutions are given in (table1) For the macronutrient stock solutions, weighing of required amount of reagent was done and transferred to a 1000 ml beaker and initial mixing was done with about 750 ml distilled water. Mixed the Solution in 2 liter volumetric flask, then add distilled water and made up volume to 2 liter. Mixed the solution properly for 15 min using a glass rod, then transfer to stock solution bottle. Preparation of micronutrient stock solution is critical because most nutrient deficiencies and other toxicities could be traced to improper preparation. Thus in micronutrient preparation considerable attention was given. Each reagent of the micronutrient solution listed in (table 1) as dissolved separately. Only ferric chloride was dissolved in 100 ml distilled water. Mix all solutions together by using 2.0 liter capacity volumetric flask. Add the ferric chloride solution to the mixture just before citric acid and mix the mixture for 15 min. Finally, 100 ml sulfuric acid was added to the mixture and volume was made up to 2.0 liter and stored in a dark glass bottle. The final color of the solution was yellowish brown. All stock solutions was properly labeled and kept separately.

Table 1: Preparation of stock solution

Element	Reagent (AR grade)	Preparation (g/4 litre solution)	Preparation (g/1 litre solution)
Macronutrient			
N	Ammonium nitrate (NH_4NO_2)	365.6	91.40
P	Sodium phosphate monobasic monohydrate ($NaH_2PO_4H_2O$)	147.4	36.85
K	Potassium sulfate (K_2SO_4)	285.6	71.40
Ca	Calcium chloride dehydrate ($CaCl_2$ $2H_2O$)	469.4	117.35
Mg	Magnesium sulfate 7-hydrate ($MgSO_4$ $7H_2O$)	1296.0	324.0
Micronutrient: Dissolved each reagent separately and mix in 2 litter distilled water then added 200ml H_2SO_4 and make up volume to 4litter			
Mn	Manganese chloride 4-hydrate (MnCl 13-4 H_2O)	8.00	2.0
Mo	Ammonium molybdate 4-hydrate [$(NH_4)_3$Mo/O24 $4H_2O$]	0.295	0.073
Zn	Zinc sulfate 7 hydrate ($ZnSO_4$ $7H_2O$)	0.110	0.027
B	Boric acid (H_2SO_4)	3.736	0.934
Cu	Cupric sulfate 5 hydrate ($CuSO_4$ $5H_2O$)	0.124	0.031
Fe	Ferric chloride 6 hydrate ($FeCl_2$ $6H_2O$)	30.800	7.7
	Citric acid monohydrate	47.600	11.9

Source: Adapted from Yoshida *et al.*, (1976)
Note: For easy handling and storage, hydrate reagents are preferred

Handling of seedlings and salinization

Test seeds have to be heat-treated for 5 days in a convection oven set at 50^0C to break seed dormancy. Proper breaking of the seed dormancy is very essential in this screening

technique. Delay in germination of some entries will likely make these entries more sensitive to salt. Seedling vigor has great advantage at this point since salinization occur at very early seedling stage. After breaking the dormancy, surface sterilize seeds with fungicide and rinse well with distilled water. Sterilized seeds were placed in petridishes with moistened filter papers and incubated at 30ºC for 48 hour for germination. Sowed two pregerminated seeds per hole on the Styrofoam seedling float. The radicle should be inserted through the nylon mesh. Suspend the Styrofoam seedling float on the tray filled with distilled water. There are adequate nutrients in the endosperm for the seedlings to grow normally for 3-4 days. After 3 days, when seedlings are well established, replaced the distilled water with salinized nutrient solution. Initial salinity is at EC = 6 dS m^{-1}. Three days later, increased salinity to EC=12 dS m^{1} by adding NaCl to the nutrient solution. Renew the solution every 8 d and maintain the pH at 5.0 daily.

Table 2: Standard Evaluation System (SES) of visual salt injury at vegetative stages

Score	Observation	Tolerance
1	Normal growth no symptoms on leaves	Highly tolerant
3	Nearly normal growth, but leaf tips or few leaves whitish and rolled	Tolerant
5	Growth severely retarded, most leaves rolled, only few were elongating	Moderately tolerant
7	Complete cessation of growth, most leaves dry, some plants dying	Susceptible
9	Almost all plants dead or dying	Highly susceptible

Results and Discussion

The rice genotypes were screened in the lab condition at pH 6.0 and EC 12 dS/m in Yoshida (1976) solution. The rice genotype scored for salinity tolerance at seedling stage based on Standard Evaluation System (SES), 1996 at 7, 14 and 21 days after salinization (Table 3). The data revealed that all the thirty rice genotypes exhibited salinity tolerance score of 1 and 3 at 7 days. At 14 days after salinization genotypes IR-68144-2B-2-2-3-166, IR-91167-133-1-1-2-3, RP-4993-55-14-3-5-1, IR-68144-2B-2-2-3-1-120, IR-91167-31-3-1-33, IR-68144-2B-2-2-3-1-127, IR-92966-95-1-3 and IR-82475-110-2-2-1-2 showed resistance to salinity; genotypes IR-64, NDR-359, IR-91175-27-1-3-1-3, IR-92953-49-1-3, MTU-1010, IR-84722-82-2-3-3-3, IR-92960-75-1-3, IR-83294-66-2-2-3-2, NUD-3, IR-91171-70-3-3, IR-91171-66-3-2-1-3, IR-91167-99-1-1-1-3, Ayaar, R-RHZ-14-7, IR-83668-35-2-2-2 and IR-91158-85-3-2-33 were recorded moderately salinity tolerance and genotypes RP-BIO-5478-185M, Taramon, R-RHZSM-4, R-RHZ-2, Sambha mansuri and Swarna showed susceptibility to salinity. At 21 days after salinization IR-68144-2B-2-2-3-166, IR-68144-2B-2-2-3-1-120 and IR-68144-2B-2-2-3-1-127 showed highly resistance to salinity; genotypes IR-91167-133-1-1-2-3, RP-4993-55-14-3-5-1, IR-91167-31-3-1-33, IR-92953-49-1-3, IR-84722-82-2-3-3-3, IR-91167-99-1-1-1-3, IR-91171-66-3-2-1-3, R-RHZ-14-7, IR-82475-110-2-2-1-2 and IR-92966-95-1-3 were recorded moderately salinity tolerance; genotypes IR-64, IR-91175-27-1-3-1-3, RP-BIO-5478-185M, IR-92960-75-1-3, NUD-3, IR-91171-70-3-3, AYAAR, IR-91158-85-3-2-33 and IR-83668-35-2-2-2 showed susceptibility to salinity and genotypes NDR-359, MTU-1010, Taramon, IR-83294-66-2-2-3-2, R-RHZSM-4, R-RHZ-2, SAMBHA MANSURI and SWARNA were Highly susceptible to salinity. An appropriate screening method that is effective in early stages of growth would potentially provide a rapid method for primary screening of large quantities of plant material. Although various screening methods under greenhouse have been proposed, but visual symptoms of salt stress may still be the most appropriate for mass screening. In this study, thirty rice genotypes were used for screening at seedling stages. The seedling stage assessment included hydroponic experiment. Salt injury score due to salinity is reflected in leaf symptoms, such as leaf rolling and burning when rice is grown under saline conditions. Rolling of the leaf tends to minimize water loss by respiration affected by water deficit. Under severe salinity, leaves showed symptoms of burning (Amirjani, 2010). In this study, injury score was depicted as criteria for salt tolerance evaluation under hydroponics. (Bayram *et al.,* 2014)

Fig 1: Showing hydrophonic condition at 7days, 14days and 21days.

Table 3: Salinity score at vegetative stage in lab condition

S. No.	Varieties	Salinity score		
		7 days	14 days	21 days
1	IR-64	1	5	7
2	IR-68144-2B-2-2-3-166	1	3	3
3	IR-91167-133-1-1-2-3	1	3	5
4	NDR-359	3	5	9
5	RP-4993-55-14-3-5-1	1	3	5
6	IR-91175-27-1-3-1-3	1	5	7
7	IR-91167-31-3-1-33	1	3	5
8	IR-68144-2B-2-2-3-1-120	1	3	3
9	IR-92953-49-1-3	3	5	5
10	MTU-1010	3	5	9
11	RP-BIO-5478-185M	3	7	7
12	Taramon	3	7	9
13	IR-68144-2B-2-2-3-1-127	1	3	3
14	IR-84722-82-2-3-3-3	3	5	5
15	IR-92960-75-1-3	1	5	7
16	IR-83294-66-2-2-3-2	3	5	9
17	R-RHZSM-4	3	7	9
18	NUD-3	1	5	5
19	R-RHZ-2	1	7	9
20	Sambha Mansuri	3	7	9
21	IR-91171-70-3-3	3	5	7
22	Swarna	3	7	9
23	IR-91171-66-3-2-1-3	3	5	5
24	IR-91167-99-1-1-1-3	1	5	5
25	Ayaar	1	5	7
26	R-RHZ-14-7	3	5	5
27	IR-82475-110-2-2-1-2	1	3	5
28	IR-92966-95-1-3	1	3	5
29	IR-91158-85-3-2-33	3	5	7
30	IR-83668-35-2-2-2	3	5	7

Conclusion

Salinity screening for thirty rice genotypes was performed at the seedling stages in the hydroponic system. Phenotyping of the germplasm was done at 6dS/m at seedling stage. Based on modified standard evaluation score for visual salt injury at seedling stage, three (IR-68144-2B-2-2-3-166, IR-68144-2B-2-2-3-1-120 and IR-68144-2B-2-2-3-1-127) genotypes were salt tolerant, ten (IR-91167-133-1-1-2-3, RP-4993-55-14-3-5-1, IR-91167-31-3-1-33, IR-92953-49-1-3, IR-84722-82-2-3-3-3, IR-91167-99-1-1-1-3, IR-91171-66-3-2-1-3, R-RHZ-14-7, IR-82475-110-2-2-1-2 and IR-92966-95-1-3) were moderately tolerant, nine (IR-64, IR-91175-27-1-3-1-3, RP-BIO-5478-185M, IR-92960-75-1-3, NUD-3, IR-91171-70-3-3, Ayaar, IR-91158-85-3-2-33 and IR-83668-35-2-2-2) were susceptible and eight (NDR-359, MTU-1010, Taramon, IR-83294-66-2-2-3-2, R-RHZSM-4, R-RHZ-2, Sambha Mansuri and Swarna genotypes were highly susceptible. The indentified salt tolerant genotypes can be potential germplam sources for future breeding program.

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