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Screening of rice (*Oryza sativa* L.) for salinity tolerance at seedling stage under hydroponic condition

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Abstract
Rice is one among the foremost suitable crop for saline soils although it's usually considered moderately sensitive to salinity. The phenotypic response of twenty rice genotypes with salt stress at EC=12 dsm^{-1} was assessed under hydroponic condition at seedling stage. The rice genotypes FL-478, IR68144-2B-2-2-3-1-120, IR-68144-2B-2-2-3-1-127 were found tolerant, while IR-91167-99-1-1- 1-3, NUD-3, NUD-2, CSR-13, IR-92953-49-1-3 and NDRK-2008 showed moderately tolerant to salinity and genotypes NDR-359, MTU-1010, TARAMON, SAMBHA MANSURI, SWARNA IR-28 and AYYAR showed vulnerable to salinity. Tolerant varieties are suggested to use as donor parents for salinity tolerance in back cross breeding and for allele mining for salinity tolerance gene/QTLs.

Keywords: QTL: qualitative trait loci, Allele: contrasting pair of gene, EC: electrical conductivity, Hydroponic condition: soil-less culture

Introduction
The plant response to salinity consists of various processes that must function in coordination to enhance both cellular hyperosmolarity and ion disequilibrium. Salt stress adversely affects agricultural yield throughout the world affecting production. Many species of higher plants, including most crops, are subjected to growth inhibition under high-NaCl condition. Rice is one among the foremost suitable crop moderately sensitive to salinity (Akbar *et al.*, 1972) [1]. It is relatively tolerant to salinity at the germination stage but its panicle initiation and pollination stage are two most salinity-sensitive growth stages which is directly related to crop yield (Heenan *et al.*, 1988, Khatun and Flower, 1995, Zeng *et al.*, 2001) [4, 5, 10]. Screening of rice genotypes at seedling stage is comparatively easier than reproductive stage and also rapid. It is very difficult at the reproductive stage (Gregario *et al.*, 1997) [2]. The typical mechanism of salinity tolerance in rice is that the exclusion or reduction of Na uptake and increased absorption of K to take care of an honest Na^+-K^+ balance within the shoot. Salt injury starts with reduction in effective leaf area. The oldest leaves start to roll then die, followed by the next older, and so on. Rice is extremely sensitive to salinity at seedling stage. Its height, root length, emergence of latest roots and dry matter decreases significantly at EC 5-6 dSm^{-1} (Pearson *et al.*, 1966, Akbar and Yabuno., 1974) [7, 1]. Salinity suppresses leaf elongation and formation of latest leaves. The screening technique is based on the ability of seedling to flourish in salinized nutrient solution.

Materials and Methods
Plant materials
Total twenty rice genotypes were used in this study, which were IR68144-2B-2-2-3-1-120, IR-68144-2B-2-2-3-1-127, IR-91167-99-1-1-1-3, IR-91167-133-1-1-2-3, NUD-3, NDR-359, IR-28, FL-478, NUD-2, CSR-13, AYYAR, NDRK-2008, IR-64, SWARNA, IR-92953-49-1-3, IR-91171-66-3-2-1-3, IR-83668-35-2-2-2, SAMBHA MANSURI, TARAMON and MTU-1010.

Screening of rice genotypes at the seedling stage

The genotypes were evaluated for tolerance to salinity in the laboratory of Department of Plant Molecular Biology and Genetic Engineering, Acharya Narendra Deva University of Agriculture and Technology, Kumar Ganj, Ayodhya using standard protocol (Gregorio *et al.*, 1997) [3]. Rice was extremely sensitive to salinity at seedling stage. Its height, root length, emergence of latest roots, and dry matter decrease significantly at EC (electrical conductivity) 5-6 dSm^{-1} (Akbar and Yabuno 1974) [1]. Salinity stressed at early seedling stage manifest on the primary leaf followed by the second and eventually on the growing leaf. Salinity suppresses leaf elongation and formation of latest leaves. Photosynthetic function and chlorophyll content were inversely proportional to salinity leveled (Ota and Yasue 1962) [6]. The screening technique developed was predicated on the power of seedlings to grow in salinized nutrient solution.

Materials and instruments required

To conduct the screening at seedling stage, need some basic equipment's 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
- Thermocol (50 and 2 cm thick for making seedling floats)
- Mixing containers: Cylindrical plastic containers, 50 liter and 100-liter capacity.

Preparation of stock solution

Proper preparation of stock solutions is important to avoid nutrient deficiencies and mineral toxicities not attributed to salinity stress. Therefore, the amounts prepared should depend upon the amount of test entries screened during a two-month period. For the macronutrient stock solutions, weighed the required amount of reagent (Table 1) and transferred to a 1000-ml beaker and initial mixing was done with 750 ml distilled water. Then mixture was transferred to 2 liter volumetric flask, then added distilled water and make up volume 2 liter. The mixture was agitated for 15 min using a magnetic stirrer then transferred to stock solution bottle. Preparation of micronutrient stock solution was critical because most nutrient deficiencies and other toxicities might be traced to improper preparation. Each reagent of the micronutrient solution listed in (Table 1) should be dissolved separately. Mixed all solutions together in distilled water using 2.0 litter capacity volumetric flask. Added the ferric chloride solution to the mixture just before citric acid and stirred the mixture for 15 min using a magnetic stirrer. Finally, added 100 ml sulfuric acid to the mixture and make up volume to 2 liter. Stirred for another 10 min and stored in a dark glass bottle. The final color of this solution was yellowish brown.

Table 1: Preparation of stock solution

Element	Reagent (AR grade)	Preparation (g/4 liter solution)	Preparation (g/1 liter 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 liter distilled water then added 200ml H_2SO_4 and make up volume to 4 liter			
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) [9].

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 ^{0}C to break seed dormancy. Proper breaking of the seed dormancy is extremely essential during this screening technique. Delay in germination of some entries will likely make these entries more sensitive to salt. Seedling vigor has great advantage at now since salinization occur at very early seedling stage. After breaking the dormancy, surface sterilized the seeds with fungicide and rinsed well with water. Sterilized seeds were placed in Petri dishes with moistened filter papers and incubated at 30 ^{0}C for 48 hours for germination. Two pre germinated seeds were sown per hole on the Styrofoam seedling float. The radicle should be inserted through the nylon mesh. Suspend the Styrofoam seedling float on the tray crammed with 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 water with salinized nutrient solution. Initial salinity is at EC = 6 dSm^{-1}. Three days later, increased salinity to EC=12 dSm^{-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 Score (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 retarted, 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 within the lab condition in Yoshida (1976) [9] solution. The rice genotypes scored for salinity tolerance at seedling stage based on Standard Evaluation System (SES), (1996) (Table 2) at 7, 14 and 21 days after salinization. The data revealed that all the varieties showed salinity tolerance with score of 3 after 7 days of salinization. Varieties IR68144-2B-2-2-3-1-120, IR-68144-2B-2-2-3-1-127, IR-91167-99-1-1-1-3, IR-91167-133-1-1-2-3, NUD-3, NDR-359, IR-28, FL-478, NUD-2, CSR-13, AYYAR, NDRK-2008, IR-64, SWARNA, IR-92953-49-1-3, IR-91171-66-3-2-1-3, IR-83668-35-2-2-2, SAMBHA MANSURI, TARAMON and MTU-1010 rice genotypes exhibited salinity tolerant with score of 3. After 14 days of salinization varieties FL-478, IR-91167-133-1-1-2-3, IR68144-2B-2-2-3-1-120, IR-68144-2B-2-2-3-1-127 showed tolerance to salinity; varieties IR-64, IR-92953-49-1-3 MTU-1010, NUD-3, IR-91171-66-3-2-1-3, IR-91167-99-1-1-1-3, AYYAR, NDRK-2008, NUD-2 and CSR-13 were recorded moderately salinity tolerance with score 5 and varieties NDR-359, TARAMON, SAMBHA MANSURI and, SWARNA showed susceptibility to salinity with score 7.

Table 3: Salinity score at vegetative stage in lab condition

S. No.	Varieties	Salinity Score		
		7 Days	14 Days	21 Days
1	IR-68144-2B-2-2-3-1-120	3	3	3
2	IR-68144-2B-2-2-3-1-127	3	3	3
3	IR-91167-99-1-1-1-3	3	5	5
4	IR-91167-133-1-1-2-3	3	3	7
5	NUD-3	3	5	5
6	NDR-359	3	7	7
7	IR-29	3	5	7
8	FL-478	3	3	3
9	NUD-2	3	5	5
10	CSR-13	3	5	5
11	AYYAR	3	5	7
12	NDRK-2008	3	5	5
13	IR-64	3	5	7
14	SWARNA	3	7	7
15	IR-92953-49-1-3	3	5	5
16	IR-91171-66-3-2-1-3	3	5	7
17	IR-83668-35-2-2-2	3	5	7
18	SAMBHA MANSURI	3	7	9
19	TARAMON	3	7	9
20	MTU-1010	3	5	9

Fig 1: Screening of genotypes under hydroponic condition during experiment.

At 21 days after salinization, FL-478, IR68144-2B-2-2-3-1-120, IR-68144-2B-2-2-3-1-127 were found tolerant, while IR-91167-99-1-1-1-3, NUD-3, NUD-2, CSR-13, IR-92953-49-1-3 and NDRK-2008 showed moderately tolerant to salinity and genotypes NDR-359, MTU-1010, TARAMON, SAMBHA MANSURI, SWARNA, IR-29 and AYYAR showed susceptible to salinity (Table 3). Gregario et al., (2002) [2] observed wide variation in for salinity tolerance in rice accessions and also reported with FL-478 and NSIC Rc222 as tolerant variant of cross Pokkali (tolerant) and IR-29 (sensitive).

Conclusion

The screening of 20 rice genotypes under hydroponics condition exhibited highly salinity tolerance score of 1 and tolerant score of 3 at 7 days. At 14 days after salinization genotypes, FL-478, IR-91167-133-1-1-2-3, IR68144-2B-2-2-3-1-120, IR-68144-2B-2-2-3-1-127 were found tolerant, while

IR-64, IR-92953-49-1-3 MTU-1010, NUD-3, IR-91171-66-3-2-1-3, IR-91167-99-1-1-1-3, AYYAR, NDRK-2008, NUD-2 and CSR-13 were recorded moderately tolerant and genotype NDR-359, TARAMON, SAMBHA MANSURI and, SWARNA showed susceptibility to salinity. At 21 days after salinization, FL-478, IR68144-2B-2-2-3-1-120, IR-68144-2B-2-2-3-1-127 were found tolerant, while IR-91167-99-1-1- 1-3, NUD-3, NUD-2, CSR-13, IR-92953-49-1-3 and NDRK-2008 showed moderately tolerant to salinity and genotypes NDR-359, MTU-1010, TARAMON, SAMBHA MANSURI, SWARNA IR-28 and AYYAR showed susceptible to salinity. The tolerant varieties are suggested for including in breeding Programme in future prospectus.

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