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Screening of different rice (*Oryza sativa* L.) genotypes for salinity tolerance at seedling stages

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Abstract: Fifteen rice genotype were screened for salinity tolerance at seedling stages in artificially saline conditions under controlled environment with EC=6 dS m^{-1} and 12 dS m^{-1} at pH=5 using visual score 1 to 9 of SES scoring. Out of fifteen rice genotype screened one was highly tolerant, five were found tolerant, four were graded as moderately tolerant and two as susceptible, three as highly susceptible. Thus we search new genotypes for better potential than Pokkali and Nonabokra recognized as salt tolerant variety, and may boost up the rice production in salt affected areas. The genetic diversity for salt tolerance parameters among fifteen genotypes is important for planning the crossing programme.

Key words: SES, EC, Salinity, Tolerance, Susceptible, Rice

Introduction

Rice (*Oryza sativa* L.) is one of the most widely cultivated crops in the world and staple food to feed more than 3 billion people and to provide 50-80% daily calorie intake (Khush *et al.,* 2005). A 100 g of rice provides 345.0 kcal, 78.2 g of carbohydrates and 6.8 g of protein (Gopalan *et al.,* 2007). Total area under salinity is about 953 million ha covering about 8% of the land surface (Singh, 2009). Nearly 6.73 million hectare soil in India is salt affected and categorized into two-broad group: Alkali soils and Saline soils. Salinity is one of the key environmental factor that limit crop growth and agriculture productivity. Abiotic stress is the main factor negatively affecting crop growth and productivity worldwide. Rice plants are relatively susceptible to soil salinity as an abiotic stress (Gao *et al.,* 2008). Breeding for salinity tolerance in rice requires reliable screening techniques. These techniques must be rapid to keep pace with the large amount of material generated for breeding. Screening under field conditions is difficult due to stress heterogeneity, presence of other soil related stress and significant influence of environmental factors such as temperature solar radiation and relative humidity. Salt tolerant breeding program in rice is a profitable issue for plant breeders. Salt tolerance is a complex, quantitative genetic character controlled by polysgenes (Singh *et al.,* 2007). Salt stress also affects many physiological aspects of plant growth witch grow in the salt affected soil tend to show differences in physiological and biochemical activities from those that grown on non salt affected soil (Lutts *et al.,* 1995). Several physiological pathways, that is photosynthesis respiration, nitrogen fixation and carbohydrate metabolism has been observed to be affected by high salinity (Chen *et al.,* 2008). Rice is tolerant during germination, becomes very sensitive during early seedling stage (2–3 leaf stage), gains tolerance during vegetative growth stage, again becomes sensitive during pollination and fertilization and then becomes increasingly more tolerant at maturity (Lutts *et al.,* 1995). Under salinity, plant has to face both osmotic and ionic stresses which ultimately cause reduction in growth (Munns and Tester, 2008). In rice the screening can be done independently at its two salt sensitive stages but screening at seedling stage is a rapid method and based on simple criteria and require less time. However, the salt tolerance at early growth stages does not always correlate with that to the subsequent growth stages (Ferdose *et al.,* 2009). Different rice varieties which yield high under normal condition; they fail to perform in salt affected soils. The genetic improvement mainly depends on the amount of genetic variability present in the population (Babu *et al.,* 2006). Hence, estimation of genetic diversity for salt tolerance parameters among genotypes is important for planning the crossing programme. The present experiment for screening of rice genotypes for salinity tolerance under controlled condition and data were recorded at seedling stage as according to SES scoring response of different rice genotypes with EC=6 dS m^{-1} and 12 dS m^{-1} at pH=5. In this experiment, we focused on evaluating the potential of salt tolerance in rice genotypes at early growth stage i.e. at seedling stage. Screening of genotypes for salt tolerance at early growth stages may be important for screening salt tolerance as a there is considerable saving time.

Materials and Methods

Sampling: Rice genotypes for screening were collected from Department of Genetics and Plant Breeding, NDUA&T, Kumarganj, Faizabad (Table-1) (Sarjoo 52, IR 64, NDR 359, Pokkali, CSR 13, CSR 90-IR 2, NDRK 5088, Narendra Usar Dhan-2, Narendra Usar Dhan-3, Nonabokra, Swarna sub-1, CSR 30, FL-478, IR 28 and IR 29). The morphological description of above 15 rice genotypes is present in table-1.

Preparation of stock solution: Stock solutions of each micro and macronutrient were prepared separately as listed in table-2 (Gregorio *et al.,* 1997). Ferric chloride dissolved in 100 mL distilled water separately. Mixed all solutions together in one litre distilled water using two litre capacity volumetric flasks. Add the ferric chloride solution to the mixture just before citric acid and stir the mixture for 20 min. Finally add 100 ml sulfuric acid to the mixture and make up volume to 2.0 liter. All stock solutions stored in a dark glass bottle separately and final color of the solution yellowish brown.

Making seedling floats and handling of seedling during salinization. The screening for salinity tolerance at seedling stage of the rice genotypes was carried on the basis of morphological characterization and visual scoring. The rectangular styrofoam seedling floats of size 28×32×1.2 cm having 5 holes for per genotype totally 75 holes (15×5) with nylon net bottom. Holes are 1.5 cm in diameter attaches nylon net on one side of styrofoam seedling floats and stich with nylon thread fit well to the top of 12 liter plastic tray. Seed were kept in oven for breaking seed dormancy; results for better and uniform germination. After breaking dormancy seeds were surface sterilization of with 0.1% $HgCl_2$ for 2-3 minutes and washed properly two to three times with distilled water. The sterilized seed in petriplates soaked in water on germination paper with for 24 hours and incubation for 48 hours at 30-32^{0}C. Pregerminated seeds were kept in hole, after germination three seed per hole and 5 holes for per genotype on styrofoam seedling floats. The radical were inserted carefully through nylon mesh. Styrofoam seedling floats suspended in distilled water a Period for 4 days, after germination, seeds were kept in non-saline distilled water for proper establishment and normal growth of seedling before they were exposed to saline nutrient solution of desired electrical conductivity (EC). The solutions with desired EC were prepared by dissolving NaCl in the nutrient solution. After 4-5 days old seedling proper established seedling floats in distilled water, replaced tray into the nutrient solution and salinized with NaCl at EC=6 dSm^{-1} and pH=5. Nutrient solution renewed weekly and its pH was maintained twice in a day at 5.0 by either adding NaOH or HCl. After one week of salinization electrical conductivity increases from EC=6 dSm^{-1} to EC=12 dSm^{-1} now visual salinity symptom were scored using modified standard evaluation system (table-3) (S.E.S., 1996).

Results and Discussions

Visual salinity symptom was scored according to modified standard evaluation system (SES) with EC=6 dS m^{-1} and 12 dS m^{-1} and at pH=5 at seedling stage. After one week of salinization, at EC=6 dSm^{-1} and pH=5 different rice genotypes showed highly tolerance to salt stress response viz. Pokkali, CSR-90-IR2, NUD-3, CSR-30, Nonabokra, FL-478, CSR-13, NDRK-5088, IR-64 and NUD-2 and tolerant were IR-28, IR-29, Sarjoo-52, NDR-359 and Swarna Sub1 presented in table-4. All the genotypes showed resistance at early stages but susceptible in later stages. Same results were observed during screening for salinity tolerance of fifteen rice genotypes at seedling and vegetative stages by Mishra *et al.* (2012). Thus most of the plant attributes either little or no effect of salinity treatment was observed during early stages. After two week of salinization i.e. 14 days scoring of rice genotypes on the basis of SES score (Table-3). The number of genotypes showed tolerance were Pokkali, Nonabokra, FL-478, CSR-30, CSR-13, NDRK-5088, IR-64, CSR-90-IR2, NUD-2, NUD-3 and moderately tolerance genotypes were NDR-359, Sarjoo-52 and susceptible were IR-28, IR-29 and Swarna Sub1. Final scoring was done at 21 days after salinization with EC=12 dSm^{-1} at pH=5, some genotypes which showed highly tolerance viz. Pokkali, CSR-30, Nonabokra and CSR-13, CSR-90-IR2, FL-478 were moderately tolerant and NUD-3, NDR-359, Sarjoo-52, NUD-2, NDRK-5088 were susceptible and IR-28, IR-29, Swarna Sub1 were highly susceptible. The highest surviving capacity during salinization showed by Pokkali and lowest IR-28, IR-29, Swarna sub-1, NDR-359 and Sarjoo-52. The NUD-3 showed better surviving capacity than NUD-2. At seventh day of salinization mostly all the genotypes showed highly tolerance to salinity viz. CSR-13, CSR-90-IR2, FL-478, Pokkali, CSR-30, Nonabokra, NUD-3, NUD-2, IR-64 and NDRK-5088. But among these, some genotypes showed tolerance type symptoms at 21 days of salinization viz. CSR-30, Nonabokra,

Table-1: Rice genotypes used in the study and their descriptions

Genotypes	Parentage	Height	Duration	Yield(q/ha)	Salinity tolerance
CSR-13	CSR-1 × Basmati-370 × CSR-5	Semi dwarf	115-130	35-40 q/ha	Moderately Tolerant
IR-28	IR 833-6-1-1-1/IR 1561-149-1/IR 1737	Semi dwarf	105-110	25-30 q/ha	Susceptible
IR-29	IR 833-6-1-1-1 (IR 1561-149-1/IR 1737)	Semi dwarf	100-110	25-30 q/ha	Susceptible
CSR-90-IR2	IR 10206-29-2-1/SUAKKO (Sel.)	Semi dwarf	115-125	35-40 q/ha	Moderately Tolerant
Narendra Usar Dhan-3	Leaungya 1148 × IR 9129-20 g-2-2-2-1 × IR 18272-27-3-1	Semi dwarf	125-130	40-45 q/ha	Moderately Tolerant
CSR-30	BR 4-10/Pak. Basmati	Tall	120-130	30-35 q/ha	Moderately Tolerant
NDRK-5088	TCCP 266-2-49-B-B-3/IR 262-43-8-11	Semi dwarf	115-125	40-45 q/ha	Moderately Tolerant
Nonabokra	Selected from India	Semi dwarf	130-135	30-35 q/ha	Tolerant
NDR-359	BG-90-2-4 × 08677 T (N) 1 × Kashi	Semi dwarf	115-125	45-50 q/ha	Susceptible
IR-64	IR-5857-33-2-1 × IR-2061-465-1-5-5	Semi dwarf	115-120	50-58 q/ha	Moderately Tolerant
Sarjoo-52	T (N) 1 × Kashi	Semi dwarf	130-133	50-60 q/ha	Susceptible
FL-478	IR29 × Pokkali	Semi dwarf	120-125	30-35 q/ha	Moderately Tolerant
Pokkali	Selection from Sri Lanka	Tall	130-140	30-35 q/ha	Tolerant
Narendra Usar Dhan-2	IRRI Line F_2	Semi dwarf	115-130	40-45 q/ha	Moderately Tolerant
Swarna Sub1	FR 13 A × IR 49830	Semi dwarf	130-140	50-60 q/ha	Susceptible

Table-2: Preparation of stock solution. Adapted from Yoshida *et al.* (1976). For easy handling and storage, hydrate reagents are preferred. Dissolve each reagent separately and mix in 2 liter distilled water then add 200 ml H_2SO_4 and make up volume to 4 liter

Elements	Reagent (AR grade)	Preparation (g/4L solution)
Macronutrient		
N	Ammonium nitrate (NH_4NO_2)	365.6
P	Sodium phosphate monobasic monohydrate (NaH_2PO_4)	147.4
K	Potassium sulphate (K_2SO_4)	285.6
Ca	Calcium chloride dehydrate ($CaCl_2.{}_2H_2O$)	469.4
Mg	Magnesium sulfate 7-hydrate ($MgSO_4.7H_2O$)	1296.0
Micronutrient		
Mn	Manganese chloride 4-hydrate ($MnCl_2.4H_2O$)	8.00
Mo	Ammonium molybdate 4-hydrate [$(NH_4)_3Mo/O_{24}.4H_2O$]	0.295
Zn	Zinc sulfate 7-hydrate ($ZnSO_4.7H_2O$)	0.110
B	Boric acid (H_3PO_4)	3.736
Cu	Cupric sulfate 5-hydrate ($CuSO_4.5H_2O$)	0.124
Fe	Ferric chloride 6- hydrate ($FeCl_2.6H_2O$)	30.800
Fe	Citric acid monohydrate ($C_6H_8O_7.H_2O$)	47.600

Table-3: 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 are elongating	Moderately tolerant
7	Complete cessation of growth, most leaves dry, some plants dying	Susceptible
9	Almost all plants dead or dying	Highly susceptible

Table -4: Salinity scoring at seedling stage (SES).

Genotype	Seedling (Day's) stage scoring			Reaction
	7	14	21	
CSR-13	1	5	5	MT
IR-28	3	7	9	HS
IR-29	3	7	9	HS
CSR-90-IR2	1	3	5	MT
NUD-3	1	3	5	MT
CSR-30	1	3	3	T
NDRK-5088	1	5	5	MT
Nonabokra	1	3	3	T
NDR-359	3	7	7	S
IR-64	1	3	5	MT
Sarjoo-52	3	5	7	S
FL-478	1	3	5	T
Pokkali	1	1	1	HT
NUD-2	1	5	5	MT
Swarna Sub1	3	7	9	HS

HT=Highly Tolerance, T=Tolerance, MT=Moderately Tolerance, S=Susceptible, HS=Highly Susceptible

Pokkali and some showed moderately tolerance viz. NUD-3, NDRK-5088, NUD-2, IR-64, and FL-478. Other genotypes at 21 days of salinization showed highly susceptible viz. IR-28, IR-29 and Swarna Sub1 but all these genotypes exhibit tolerance at early stage that is 7 days of salinization. The mapping population by hybridizing IR 28 with Pokkali to tag salt tolerant gene(s). Phenotyping of F_3s and parents were done under NaCl (12 dSm^{-1}) stressed condition were Pokkali showed tolerance and IR-28 having extreme sensitivity to salinity (Rajani *et al.,* 2010). Rice plants are very sensitive to salinity during the seedling stage (Pearson and Bernstein, 1959 and Flowers and Yeo, 1981). Screened *Basmati* rice varieties for salt tolerance and characterize genetic diversity among the rice varieties with different adaptations to saline soils using Yoshida solution containing 0 mM NaCl (control, pH 5.0) and 30 mM NaCl (Electrical conductivity 4.8 d/S, pH 5.0) and assessed for salinity tolerance on 1–9 scale as per IRRI standard evaluation system using seedling growth parameters, visual salt injuries, Na^+/K^+ ratio and microsatellite (SSR) and ISSR markers (Yadav *et al.,* 2008).

At present, work is going on to find out salinity tolerance and susceptible genotypes could be distinguished conveniently with EC=6 dSm^{-1} to EC=12 dSm^{-1} at pH=5 by visual salinity symptom were scored using modified standard evaluation system (SES, 1996). Thus in this study found Pokkali, Nonabokra, CSR-13, CSR-90-IR2, CSR-30, NUD-3, NUD-2 and FL-478, recognized salt tolerant variety and may boost up the rice production in salt affected areas.

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