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Screening of breeding lines of Improved Samba Mahsuri possessing *Saltol* QTL for seedling stage salinity tolerance

Rekha G, Senguttvel P, Kousik MBVN, Prasanna B, Swapnil K, Punniakoti E, Anila M, Ayyappa D, Pragya S, Ravinder K, Hajira SK, Balachandran SM and Sundaram RM

Rekha G
Indian Institute of Rice Research (ICAR-IIRR), Hyderabad, Telangana, India

Senguttvel P
Indian Institute of Rice Research (ICAR-IIRR), Hyderabad, Telangana, India

Kousik MBVN
Indian Institute of Rice Research (ICAR-IIRR), Hyderabad, Telangana, India

Prasanna B
Indian Institute of Rice Research (ICAR-IIRR), Hyderabad, Telangana, India

Swapnil K
Indian Institute of Rice Research (ICAR-IIRR), Hyderabad, Telangana, India

Punniakoti E
Indian Institute of Rice Research (ICAR-IIRR), Hyderabad, Telangana, India

Anila M
Indian Institute of Rice Research (ICAR-IIRR), Hyderabad, Telangana, India

Ayyappa D
Indian Institute of Rice Research (ICAR-IIRR), Hyderabad, Telangana, India

Pragya S
Indian Institute of Rice Research (ICAR-IIRR), Hyderabad, Telangana, India

Ravinder K
Indian Institute of Rice Research (ICAR-IIRR), Hyderabad, Telangana, India

Hajira SK
Indian Institute of Rice Research (ICAR-IIRR), Hyderabad, Telangana, India

Balachandran SM
Indian Institute of Rice Research (ICAR-IIRR), Hyderabad, Telangana, India

Sundaram RM
Principal Scientist (Biotechnology), Indian Institute of Rice Research (ICAR-IIRR), Hyderabad, Telangana, India

Correspondence
Sundaram RM
Principal Scientist (Biotechnology), Indian Institute of Rice Research (ICAR-IIRR), Hyderabad, Telangana, India

Abstract

The present investigation was carried out to evaluate the seedling stage salinity tolerance of 12 breeding lines developed in the genetic background of Improved Samba Mahsuri (ISM), possessing the *Saltol* QTL using FL478 as tolerant check by adopting IRRI standard protocol (hydroponics). The tolerance levels were assessed after 12 days of exposure to four different levels of saline solution (with electrical conductivity of 4, 6, 8, 10, 12 dS m $^{-1}$). While, all the twelve breeding lines were observed to be tolerant to seedling stage salinity, three lines, viz., i.e. ISM-18-217-18-141, ISM-110-39-23-147, ISM-105-34-6-182 showed a higher degree of tolerance with a phenotypic score of 1 and five other lines, viz., ISM-100-17-66-98, ISM-84-7-12-47, ISM-10-12-67-157, ISM-102-179-13-179, ISM-19-15-178-142 showed a score of 3, while four lines i.e., ISM-9-5-50-160, ISM-22-102-179-143, ISM-3-181-90-196, ISM-82-79-45-76 exhibited a score of 3. The development of breeding of lines of Improved Samba Mahsuri possessing tolerance to seedling stage salinity can be helpful to increase the income of farmers living in areas affected by coastal and inland salinity.

Keywords: rice, salinity tolerance, hydroponics, SES, seedling stage

Introduction

Rice is the staple food of more than 50% of the world's population (Aggarwal *et al*., 2002) [1]. By the year 2025, 21% increase in rice production will be needed over that of year 2000 (Bhuiyan *et al*., 2002) [3]. It provides almost 50–80% of daily calorie intake amongst the poor class of the society. It's a staple food and cash crop for more than three billion people in the World (Ma *et al*., 2007) [12]. Asian farmers constitute about 92% of the world's total rice producing group (Mitin 2009). In Asia 90 % of rice is produced by small farmers who are solely dependent on rice for their livelihood and food security (ANU 2006).

Salinity is one of the major obstacles in increasing production in rice growing areas worldwide, which is an ever-present threat to crop yield. Therefore, development of salt tolerant varieties has been considered as one of the strategies to increase rice production in saline prone coastal areas. The response of rice to salinity varies with growth stage. Several studies indicated that rice is tolerant during germination, becomes very sensitive during early seedling stage (2-3 leaf stage), gains tolerance during vegetative growth stage, becomes sensitive during pollination and fertilization and then becomes increasingly more tolerant during grain maturity (IRRI, 1967). As per the classification of tolerance to salinity, rice is within the sensitive division from 0 dS m −1 to 8 dSm −1 (Mass 1986) [13]. Quijano-Guerta and Kirk 2002 reported that the cheapest and easiest way to address the problem of salinity is through the development of a salt tolerant crop variety. For this, the foremost step is to screen the existing gentotypes to identify the potential breeding materials. Screening at field level proved to be difficult due to soil heterogeneity, climatic factors and other environmental factors which may influence the physiological processes. Hence, screening under laboratory conditions is considered to be advantageous over field screening. International Rice Research Institute has developed a high-throughput method based on hydroponic culture for screening salinity tolerance/susceptibility at seedling stage (Gregorio *et al*., 1997) [7]. Further, a major QTL associated with tolerance to salinity at seedling stage, viz., *Saltol* has been discovered from the Indian saline tolerant landrace, Pokkali and the QTL has been fine mapped.

The aim of the present study was to screen breeding lines of Improved Samba Mahsuri possessing the major QTL associated with seedling stage salinity tolerance, i.e. *Saltol* under salinized and non-salinized conditions in hydroponics to evaluate the salt tolerant genotypes at the seedling stage when exposed to different salinity treatments.

Materials and Methods

Plant material: Twelve breeding lines possessing seedling stage salinity tolerance QTL *Saltol* in the genetic background of Improved Samba Mahsuri (ISM) were investigated for salinity tolerance at seedling stage at ICAR-Indian Institute of Rice Research (IIRR) glasshouse under controlled conditions. FL478 was used as tolerant check while ISM as a susceptible check.

Seed germination: For each genotype, sterilized seeds were placed in Petri dishes with moistened blotting paper and incubated for 48 hours to germinate (Gregorio *et al*., 1997) [7]. These pre-germinated seeds were then transferred to the styrofoam seedling float for evaluation under salinity stress.

Establishment of seedlings for screening

The experiment was conducted in the glass house of ICAR-IIRR maintained at 30/20 °C day/night temperatures with 70 % humidity and 16 h photoperiod. At seedling stage, genotypes were screened for salt tolerance in a hydroponic system using International Rice Research Institute's (IRRI) standard protocol (Gregorio *et al*., 1997) [7]. Three replications were used in the experiment along with tolerant (FL478) and susceptible (ISM) checks. Salinized and non-salinized sets were maintained. The evaluation was performed using the nutrient solution proposed by Yoshida *et al*., 1976 [23]. Two pre-germinated seeds were planted per hole on the Styrofoam seedling float. Each replicate consisted of 8 such holes. The radicle was inserted through the nylon mesh carefully without damaging the radical which interferes with inherent of salt tolerance mechanism of rice. Therefore, seedlings were allowed 3 days to repair for any root damage. After the recovery seedlings were allowed to grow under normal nutrient solution up to 7 days. After well establishment of seedlings, nutrient solution was salinized by adding different concentrations of NaCl i.e. 40mM, 60mM, 80mM, 100mM and 120mM (electrical conductivity of 4, 6, 10, 12 dS m $^{-1}$). Due to evaporation and transpiration there was a loss of solution volume changing the P^{H}, the volume was adjusted back to the appropriate levels for every two days and the pH was adjusted to 5 using 1% HCl or 5 % NaCl. Solution was changed by lifting off the Styrofoam seedling float and placing them temporarily onto an empty basin and pouring the hydroponics solutions back into a big container where the bulked solution is pH adjusted for the whole experiment in one step. Once adjusted, the solution was re-distributed into the test containers and the seedling platform returned. These operations also helped to aerate the hydroponics solution.

Evaluation of rice genotypes at seedling stage: Visual symptoms of salinity stress includes reduced leaf area, whitish appearance of lower leaves, leaf tip death and leaf rolling. The modified standard evaluation system (SES) was used in rating the visual symptoms of salt toxicity (IRRI 2013). Scoring of salinity tolerance was performed as shown in Table 1

Table 1: Modified standard evaluation score (SES) of visual salt injury at seedling stage

Score	Observation	Tolerance
1	Normal growth, no leaf symptoms	Highly tolerant
3	Nearly normal growth, but leaf tips or few leaves whitish and rolled	Tolerant
5	Growth severely retarded; most leaves rolled; only a 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

Discriminating the susceptible ones from the tolerant and the moderately tolerant genotypes. Initial and final scorings were performed 15 days and 21 days after salinization, respectively. At this stage, sensitive seedlings begin to die, whereas test entries show varying degrees of tolerance gives classification criteria for salt tolerance based on the known standards.

Results

Twelve genotypes showed wide variation in phenotypic screening under salinity stress. Seedlings grown in salinized condition showed different visual symptoms of salt injury while the seedlings under non salinized conditions were healthy and with no symptoms. The symptoms were prominent on the first and second leaves and were visualized by leaf rolling, formation of new leaf, brownish and whitish of leaf tip, drying of leaves and also by reduction in root growth, stunted growth and stem thickness leading to complete cessation of growth and dying of seedlings.

Salt tolerant seedlings were distinguished from the sensitive seedlings grown in salinized condition after 15 days for initial scoring and after 21 days for final scoring based on the visual symptoms of sensitive and tolerant checks. The genotypes were scored 1 (highly tolerant) to 9 (highly susceptible) using modified standard evaluation system (SES) of IRRI 2013 for rating the visual symptoms of salt injury (Table 2; Figure 1) with respect to the tolerant cultivar FL478.

Table 2: Phenotypic scoring of genotypes based on IRRI SES 2013

S. No	codes	Salt injury scores	Tolerance
1	FL478	1	HT
2	RPBio-226	9	S
3	ISM-9-5-50-160	7	MT
4	ISM-19-15-178-142	3	T
5	ISM-18-217-18-141	1	HT
6	ISM-22-102-179-143	7	MT
7	ISM-102-179-13-179	3	T
8	ISM-110-39-23-147	1	HT
9	ISM-3-181-90-196	7	MT
10	ISM-82-79-45-76	7	MT
11	ISM-10-12-67-157	3	T
12	ISM-84-7-12-47	3	T
13	ISM-105-34-6-182	1	HT
14	ISM-100-17-66-98	3	T

*HT- Highly tolerant; MT-Moderately tolerant; T-Tolerant check; S-Sensitive check

In the present study, the seedlings were treated at different concentrations of NaCl i.e., 40mM, 60mM, 80mM, 100mM and 120mM. It was observed that no symptoms were developed when treated at 40mM and 60mM concentration of NaCl and genotypes along with checks were healthy. At

80mM and 100mM concentration, slight differences in the plant growth were observed along with visual symptoms of stress after four weeks of exposure in susceptible and tolerant checks.

However, clear differences were observed in the trays exposed at 120mM concentration after 15 days and 21 days. Scoring was done initially after 15 days and finally after 21 days. Out of 12 genotypes, 3 were identified as highly tolerant with 1 score (ISM-18-217-18-141, ISM-110-39-23-147, ISM-105-34-6-182); 5 as tolerant with 3 score (ISM-100-17-66-98, ISM-84-7-12-47, ISM-10-12-67-157, ISM-102-179-13-179, ISM-19-15-178-142) and 4 were identified as moderately tolerant (ISM-9-5-50-160, ISM-22-102-179-143, ISM-3-181-90-196, ISM-82-79-45-76) with score 5 (Table 2).

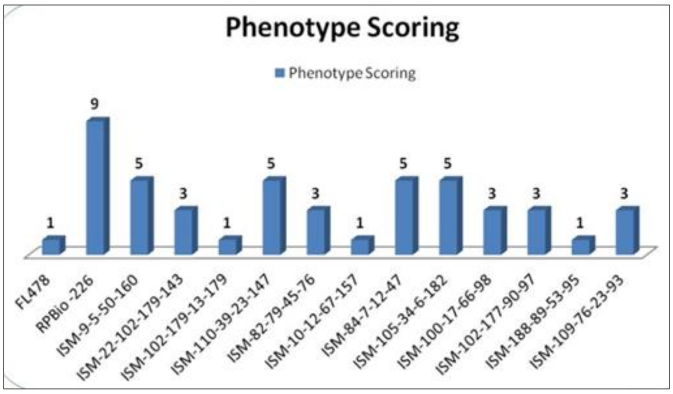

Fig 1: Schematic representation of phenotypic evaluation of genotypes for seedling stage salinity tolerance at 120mM NaCl concentration

Discussion

Salinity is the second most widespread soil problem in rice growing countries after drought and is considered as a serious limitation to increase rice production worldwide (Gregorio 1997) [7]. It causes yield reduction and also shrinks caloric and potential of agricultural products (Yokoi *et al*. 2002) [22] causing leaf injury or death of plants, thus exceeding the capacity of salt compartmentalization in cytoplasm (Munns *et al*. 2006) [17]. Several studies indicated that 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) [11]. Munns *et al*. (1982) [16] reported that salinity might directly or indirectly inhibit cell division and enlargement in the plant's growing period. Reduced shoot growth caused by salinity originates in growing tissues, not in mature photosynthetic tissues. As a result, leaves and stems of the affected plants appear stunted. The determination of salinity sensitive parameters under salinity stress helps in developing better management practices for growing rice under salinity and improves understanding of salt tolerance in rice.

However, conventional methods of screening for salt tolerance are not easy because of environmental effects and narrow sense heritability of salt tolerance (Gregorio 1997) [7]. This hinders the development of an accurate, rapid and reliable screening technique. 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. In vegetative stage root length, shoot length and biomass have been proved as the potential indicators for screening of salt tolerance (Akbar *et al*. 1986; Jones 1986; Yeo and Flowers 1986; Flowers and Yeo 1995) [2, 6, 21]. It has also been reported that the assessment of the actual salt tolerance of the genotypes may be determined by comparisons of their biomass production only after a long growth period (Leland *et al*. 1994); which therefore serves as another criterion to evaluate the salt tolerance. However, the salt tolerance at early growth stages does not always correlate with that to the subsequent growth stages (Mass and Grieve 1994; Zeng *et al*. 2002; Ferdose *et al*. 2009) [14, 5]. In this study, therefore, 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 stages may be important for screening salt tolerance as there is considerable saving in time.

Tolerant cultivars showed less growth reduction than sensitive genotypes under salinized conditions (Qian *et al*., 2001; Suplick-Ploense *et al*., 2002) [18]. In present study considerable effects due to salinity were observed in breeding lines of ISM. Salinity response to rice occurs in varying degrees at all growth stages from germination to maturity. The genotypes along with checks when exposed at different salt concentrations (40mM, 60mM, 80mM, 100mM and 120mM), no symptoms were observed at 40mM and 60mM concentration and seedlings were healthy. Some differences in growth of seedlings and visual symptoms of salt stress were observed at 80mM and 100mM NaCl concentration after four weeks of exposure. Clear differences in the symptoms were observed when exposed at 120mM concentration. Out of 12 genotypes; 3 were identified as highly tolerant, 5 were tolerant and 4 genotypes were moderately tolerant. Foolad (1999) found that only a few major QTLs could account for a large portion of the total phenotypic variation for salt tolerance. Salt stress affected the yield and yields components both at seedling and reproductive stages. Selected salt tolerant genotypes could be used as a donor for transferring tolerant genes into other desirable cultivars to develop salt tolerant high yielding cultivars and also used for cultivation under saline prone areas.

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References

1. Aggarwal RK, Shenoy VV, Ramadevi J, Rajkumar R, Singh L. Molecular characterization of some Indian Basmati and other elite rice genotypes using fluorescent – AFLP. Theor. Appl. Genet. 2002; 105:680-690.
2. Akbar M, Khush GS, HilleRisLambers D. Genetics of salt tolerance in rice. Proceedings of the Rice Genetics. International Rice Research Institute, Manila, Philippines, 1986, 399-409.
3. Bhuiyan MAR. Efficiency in evaluating salt tolerance in rice using phenotypic and marker assisted selection. M.Sc. dissertation, Department of Genetics and Plant Breeding, Bangladesh Agricultural University, My men Singh, Bangladesh, 2005, 96.
4. ANU. Technological Transformation of Productivity, Profitability and Sustainability: Rice. In The First Ten K

R Narayanan Orations: Essays by Eminent Persons on the Rapidly Transforming Indian Economy. Australia South Asia Research Centre The Australian National University, 2006. http://epress.anu.edu.au/narayanan/mobile_ devices/pr01.html.

5. Ferdose J, Kawasaki M, Taniguchi M, Miyake H. Differential sensitivity of rice cultivars to salinity and its relation to ion accumulation and root tip structure. Plant Prod Sci. 2009; 12:453-461.
6. Flowers TJ, Yeo AR. Breeding for salinity resistance in crop plants: where next? Aust J Plant Physiol. 1995; 22:875-884.
7. Gregorio GB. Tagging salinity tolerance genes in rice using amplified fragment length polymorphism (AFLP). PhD. thesis, University of the Philippines, Los Baños. 1997; 118.
8. IRRI (International Rice Research Institute). Annual Report for Los Baños, Laguna, Philippines. 1967; 308.
9. Jones MP. Genetic analysis of salt tolerance in mangrove swamp rice. Proceedings of the Rice Genetics. International Rice Research Institute, Manila, Philippines.1986; 411-422.
10. Leland EF, Catherine MG, Eugene VM, Scott ML. Time of salt stress affects growth and yield components of irrigated wheat. Agron J. 1994; 86:100-107.
11. Lutts S, Kinet JM, Bouharmont J. Changes in plant response to NaCl during development of rice (*Oryza sativa* L.) varieties differing in salinity resistance. J Exp Bot 1995; 46:1843-1852.
12. Ma HL, Zhu JG, Liu G, Xie ZB, Wang Y, Yang L, Zeng Q. Availability of soil nitrogen and phosphorus in a typical rice–wheat rotation system under elevated atmospheric [CO2]. Field Crop Res. 2007; 100:44-51.
13. Mass EV. Salt tolerance of plants. Appl Agri Res. 1986. 1:12–26.
14. Mass EV, Grieve CM. Tiller development in salt-stressed wheat. Crop Sci. 1994; 34:1594-1603.
15. Mitin A. 2009. Documentation of selected adapted strategies to climate change in rice cultivation. East Asia Rice Working Group. 2009; 25-28.
16. Munns R, Greenway H, Delane R, Gibbs J. Ion concentration and carbohydrate status of the elongating leaf tissue of *Hordium vulgare* growing at high external NaCl. II. Cause of the growth reduction. J Exp. Bot. 1982; 33: 574-583.
17. Munns R, James RA, Läuchli A. Approaches to increasing the salt tolerance of wheat and other cereals. J Exp Bot. 2006; 57:1025-1043.
18. Qian YL, Koski AJ, Welton R. Amending isolite and zeolite in sand under saline conditions: leachate composition and salt deposition. Hort Science. 2001; 36:717-720.
19. Quijano-Guerta C, Kirk GJD. Tolerance of rice Germplasm to salinity and other chemical stresses in tidal wetlands. Field Crop Res. 2002; 76:111-121.
20. Suplick-Ploense MR, Qian YL, Read JC. Salinity tolerance of Texas bluegrass, Kentucky bluegrass and their hybrids. Crop Sci. 2002; 42: 2025-2030.
21. Yeo AR, Flowers TJ. Salinity resistance in rice (*Oryza sativa L.*) and a pyramiding approach to breeding varieties for saline soils. Effect of drought on plant growth. In: Turner NC, Passioura JB (eds) Salts in soils. CSIRO, Melbourne, Australia, 1986; 161-173.
22. Yokoi S, Bressan RA, Hassegawa PM. Salt Stress tolerance of Plant. JIRCUS Working Report. 2002; 25-33.
23. Yoshida S, Forno DA, Cook JH, Gomez KA. Laboratory manual for physiological studies of rice. International Rice Research Institute (IRRI), Los Baños, Laguna, Philippines. 1976; 61-66.
24. Zeng L, Shannon MC, Grieve CM. Evaluation of salt tolerance in rice genotypes by multiple agronomic parameters. Euphytica. 2002; 127:235-245.