

PHENOTYPING RICE GERMPLASM ASSOCIATED WITH SALINITY TOLERANCE UNDER HYDROPONICS SYSTEM

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Abstract

Screening of different rice (*Oryza sativa* L.) germplasms or breeding lines is a continuous effort to identify the promising source. A series of experiments (20) were undertaken to identify promising materials for five years of salinity screening from 2015 to 2019. The materials included total of 3,195 rice germplasm and breeding lines, out of which Bangladesh Rice Research Institute (BRRI) developed 2, 295 germplasms, 193 advanced breeding lines from BRRI, and 707 advanced breeding lines from International Rice Research Institute (IRRI), Philippines. From this study, the genotypes were categorized as 122 tolerant, 220 moderately tolerant, 1,207 sensitive, and 1,646 highly sensitive. Among the BRRI germplasms, most of the materials (61%) had sensitive responses against salinity, while only 2 and 3% of rice germplasm exhibited tolerant and moderately tolerant, respectively. BRRI lines comprised 44% sensitive and 35% highly sensitive rice genotypes. In the tolerance level, 4% appeared as tolerant and 17% moderately tolerant against salinity. Likewise, IRRI lines also showed relatively higher tolerance (9%) than the BRRI germplasm and lines. They were classified into 9% tolerant and 16% moderately tolerant rice genotypes.

Introduction

Among abiotic stresses, salinity is the second most destructive constraint on rice production after drought, affecting approximately 1 billion hectares of land worldwide (Fageria *et al.*, 2012). Rice is relatively more sensitive to salt stress in seedling and early vegetative stages (Lutts *et al.*, 1995; Mondal *et al.*, 2020) and later at the reproductive phase (Singh *et al.*, 2010). Sodium chloride (NaCl) is dominant in saline soils among many salt contaminants and is readily soluble in water to yield toxic ions like sodium (Na⁺) and chloride (Cl⁻). Roots of higher plants absorb readily smaller molecule, Na⁺, and ultimately translocate to all plant parts causing ionic imbalance, osmotic stress, and nutritional imbalance in rice plants (Siringam *et al.*, 2011; Barua *et al.*, 2015). Direct salt accumulation disrupts metabolic processes and all major morpho-physiological and yield-related features, including tiller number, panicle length, spikelet number per panicle (Khatun *et al.*, 1995), grain filling (Rao *et al.*, 2013), plant biomass (Zeng *et al.*, 2007), and photosynthesis (Baker, 2008), resulting drastically yield reduction. An essential tool for managing the rice field's salinity problem is integrating plant tolerance and cultural practices. Therefore, development of salt-tolerant high-yielding rice genotypes can be the best attempt to cultivate salinity-affected areas (Hakim *et al.*, 2014).

Rice landraces play a prominent role in attaining local food security and serve as an excellent genetic reserve for rice genomics upgrading (Tang *et al.*, 2002). Screening of rice germplasm for salt tolerance at the early seedling stage relies on genetic potentiality and seedling vigor under salinity stress. To widen the genetic base, the reliable use of the best sources, identifying a larger number of genotypes

under salt stress is necessary. Thus, the screening program was undertaken to determine performances of different rice germplasm and advanced breeding lines of both BIRRI and IRRI against salt stress that can be used as donors in the salinity tolerance breeding program.

Materials and Methods

Plant materials

Seeds of rice genotypes were received from Plant Breeding Division, Genetic Resource and Seed Division, Biotechnology Division and Hybrid Rice Division, BIRRI, Bangladesh and IRRI, Philippines. An initial evaluation of 3,258 rice genotypes consisting of 2295 Bangladeshi germplasm (mostly old indigenous genotypes), 193 advanced breeding lines developed by BIRRI, 707 advanced breeding lines introduced from IRRI and 62 line/variety from other sources was conducted. Beside, Pokkali, Nona Bokra and IR 58443-6B-10-3 were used as tolerant check variety and IR29 and IRRI 154 were used as salt-sensitive check varieties.

Experimental condition

The experiment was done at the greenhouse of the Plant Physiology Division, BIRRI from 2015 to 2019. All the rice germplasms were screened at the seedling stage for salt tolerance in the hydroponics system using IRRI standard protocol (Gregorio *et al.*, 1997). At first, seedlings were raised in the hydroponic system using Yoshida nutrients solution with EC level 12 dSm⁻¹ under the laboratory conditions. Yoshida nutrient solution comprised macronutrients and micronutrients and was prepared as suggested by Yoshida *et al.* (1976).

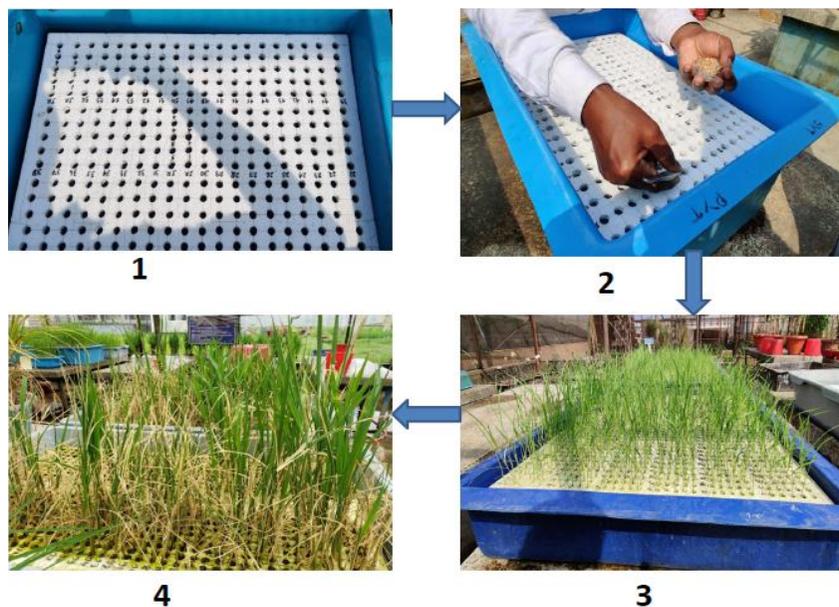


Fig. 1. Rapid screening technique for rice genotypes under salinity stress in hydroponic systems using plastic trays (1= Styrofoam ready for seed sowing, 2 = Seed sowing, 3 = Seedling growing in saline water 4 = experiment is ready for salinity scoring).

The screening of rice genotypes for salt tolerance at the seedling stage was conducted in the hydroponics system (Gregorio *et al.*, 1997), which is termed as a rapid screening technique that uses plastic trays with tight-fitting thermocol support platform with holes for placing the seeds (Figure 1). Seeds were sown in the nylon mesh set just below the thermocol platform. Before the greenhouse experiment, the seeds were disinfected with NaOCl solution (200 ml NaCl in 1-litre water) and incubated for 48 hours to enhance germination. One pre-germinated seed was sown in each hole of

Styrofoam seedling float and the seedling floats were then covered with a lid for 2 to 3 days to promote germination in the dark. The tap water was replaced with Yoshida nutrient solution after three days of seeding. Salinization was done seven days after sowing. After seven days, salinity was developed by adding crude salt to obtain an EC of 12 dSm⁻¹. The Yoshida solution's volume was adjusted to touching the seedling float at two days intervals. The pH was adjusted to 5 and EC was also adjusted with 12 dSm⁻¹ synchronizing with the Yoshida solution. When EC was higher than 12 dS m⁻¹, tap water was applied to the solution, and NaCl was added when EC was lower than 12 dS m⁻¹. Alikely, 1 N HCL was added to the solution when it's pH was more than 5.0 and 1 N NaOH was added when pH was less than 5.0.

Scoring of rice germplasms and breeding lines

Based on the visual symptoms like reduced leaf area, changing of lower leaves into whitish color, leaf tip death and leaf rolling, the rice seedlings were categorized as highly tolerant, tolerant, moderately tolerant, sensitive and highly Sensitive (Table 1). The standard saline tolerant genotypes, Pokkali, Nona Bokra, IR 58443-6B-10-3 and saline sensitive genotype IR29 and IRRI 154 were used for test comparison. Plants were exposed to salinity until the sensitive check variety died. After that rice genotypes were compared with both sensitive and tolerant check variety. If the phenotypes of any plants close to tolerant check variety were identified and the tested genotype as tolerant genotypes close to sensitive check variety e identified the tested genotype as sensitive genotype. The visual scoring for salinity tolerance was done by Standard Evaluation System (SES) score developed by Gregorio *et al.* (1997).

Experimental Design and analysis

Experimentals were carried out by following RCBD design with three replications. Data analyses were performed using Statistical Tool for Agricultural Research (STAR), version 2.0.1, developed by International Rice Research Institute (STAR, 2013).

Table 1. Modified standard evaluation score (SES) of visual salt injury at seedling stage (Gregorio *et al.*, 1997)

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 drying	Sensitive
9	Almost all plants dead or dying	Highly sensitive

Results

A total of 3,195 rice germplasms were screened during five-years-period, 2015–2019 (Table 2). Out of these, 2,295 sources, germplasm were collected from Genetic Resources and Seed Division, BRRI, 193 advanced lines from the Plant Breeding Division, BRRI and 707 advanced lines from the IRRI. The response of the rice varieties against salinity is presented in Table 2.

Table 2. The number of rice genotypes rated for salinity tolerance in BRRI, Gazipur, Bangladesh from 2015-2019

Sources	Response to salinity				Total
	T	MT	S	HS	
BRRI germplasm	48	72	774	1,401	2,295

BRRi lines	8	33	84	68	193
IRRI lines	66	115	349	177	707
Total	122	220	1,207	1,646	3195

T = tolerant; MT= moderately tolerant; S = sensitive; HS = highly sensitive

From the above study, 125 tolerant, 225 moderately tolerant and 1,245 sensitive and 1,662 highly sensitive germplasms were identified from four different rice germplasm sources.

Of the 2,295 genotypes of BRRi germplasm, 1401 were highly sensitive, and only 48 entries were tolerant to salinity stress (Table 2). In BRRi lines, the higher number of rice genotypes (84) was sensitive, and only eight entries were found tolerant to salinity from a total of 193 advanced breeding lines. Similarly, 349 rice genotypes were scored as sensitive and 66 genotypes exhibited tolerance to salinity from 707 advanced breeding lines developed in IRRI.

The percentage of tolerant, moderately tolerant, sensitive, and highly sensitive rice genotypes varied greatly among three different sources of rice genotypes, including BRRi germplasm, BRRi lines, IRRI lines, and others (Figure 2).

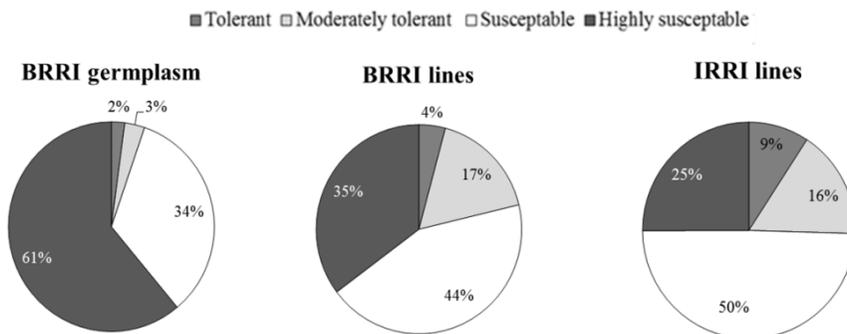


Fig. 2. Percentage shared by each source of rice genotype groups, including BRRi germplasm (n=2295), BRRi lines (n=193), IRRI lines (n=707).

Most of the BRRi germplasms are 40 sensitive (34%) and highly sensitive (61%) to salinity stress and only 2 and 3% of genotypes were tolerant and moderately tolerant, respectively. On the other hand, BRRi developed advanced breeding lines comprised of 44% sensitive and 35% highly sensitive. In terms of tolerance, 4% appeared as tolerant, and 17% moderately tolerant against salinity stress.

Like BRRi germplasm and BRRi lines, IRRI lines also had a higher percentage of sensitive (50%) materials. But IRRI lines had relatively higher tolerance with a figure of 9% tolerant and 16% moderately tolerant rice genotypes. Taking into account, BRRi lines and IRRI lines had higher cumulative frequency of tolerant genotypes ranging from 21 to 25% tolerant and moderately tolerant rice genotypes.

The potentiality of different sources of rice genotypes against salinity stress

In the present study, screened 3,195 rice genotypes for salinity tolerance. In each group of genotypes-sources, most of the materials were found sensitive to salinity stress with very few tolerant genotypes. Out of 2,295 germplasms, only 48 rice genotypes scored three (3) based on the performance against salinity stress in greenhouse conditions (Table 3).

Table 3. List of 48 rice genotypes from BRRi germplasm with SES score 3 evaluated during 2015 to 2019 at BRRi, Gazipur, Bangladesh

Name of the genotypes	SES
Hashakumira, Bhobanibhog, Bhawalia, Kumari amon, Kolom, Lal kumari, Pura binni,	3

Kajal kori, Binni, Khama rang, Temboro, Lembur, Chan moni, Murali (2), Beti balam, Patnai (3), Lati sail, Nedukenari, Raja morol, Kajol gouri, Jamrishaity, Ausbaku, Kachra boron, Modu sail, Binna chupi, Ful kumari, Raja sail tapl -14, Radha, Gutiswana, Fazla (nawgan), Kajal (nawgon), Dudkalam, Sadamota, Lamba vojon, Kali binni, Kalobirun, Boainchabirum, Moinamoti, Boleshwas, Bambudhan, Changaidhan, Kalo sail, Gaindha, Hijoldhiga, Munsur, Nona khorchi, Hoglapata, Benapol(brown)

From a total of 193 BRRI lines developed at BRRI, only eight advanced breeding lines rated score (SES) three (3) based on screening results against salinity stress in greenhouse conditions (Table 4). Among the tested genotypes, most of them were sister lines and originated from the same crosses.

Table 4. List of 8 rice genotypes from BRRI lines with SES score 3 in evaluations conducted from 2015 to 2019

Name of the genotypes	SES
BR7091-4R-1, BR7098-4R-2, BR7100-R-6-6, BR7102-4R-1, BR7105-4R-2, BR7109-5R-4, BR7113-4R-1, BR9539-B-12-11-13	3

Like BRRI lines, IRRI lines also showed potentiality with a better performance against salinity stress. Sixty-six advanced breeding lines ranked score (SES) three (3) based on screening results against salinity stress in greenhouse conditions from a total of 707 IRRI lines produced at IRRI, Philippines (Table 5).

Table 5. List of 66 rice genotypes from IRRI lines with SES score 3 conducted during 2015 to 2019 at BRRI, Gazipur, Bangladesh

Name of the genotypes	Source
IR15T1409, IR15T1473, IR90477-74-1-2-3, IR106469-23-3-AJY1-B-1, IR108174-B-CMU 10-1-B-1, IR126952-1699-41-8-10-9, IR126952-28-55-9-3-15-B, IR126952-29-12-508-10-5, IR126952-29-27-58-1-2, IR126952-29-85-275-20-1, IR66946-3R-178-1-1, IR89330-14-3-12-2-3, IR89331-32-3-1-3-2-2, IR90477-74-1-2-3-2-AJY, IR91669-16-3-2-2-2, IR92860-33-CMU 1-1CMU 2-AJY B, IR09T484, IR87916-4-1-2-1-1-B, IR87938-1-1-1-2-1-3-B, IR87938-1-1-3-2-1-B, IR87938-1-2-2-1-3-B, IR87938-1-2-2-2-1-B, IR15T1324, IR15T1408, IR15T142, IR15T1466, IR58443-6B-10-3, IR63307-4B-4-3, IR65833-4B-17-1-3, IR66946-3R-178-1-1, IR69992-AC2, IR69997-AC2, IR70870-B-P-25-2, IR72579-B-3-2-3-14, IR72579-B-3-2-3-8, IR72593-B-13-3-3-1, IR72593-B-18-2-2-2, IR72593-B-3-2-2-2, IR77674-5-1, IR83416-7-B-12-3-1-3-AJYI-B, IR87831-3-1-1-2-2-BAY- B, IR87870-6-1-1-1-1-B, IR87872-7-1-1-2-1-B, IR87888-3-AJYI-B, IR14T106, IR15T1074, IR15T1106, IR15T1107	3

Discussion

In rice, significant genetic diversity in salt stress tolerance is available. Combining superior alleles from various sites, the availability of multiple breeding tolerance sources and gene exploration can further extend the genetic base and increase the degree of tolerance (Rahman *et al.*, 2016). Identified 48 new potential donors for salt tolerance. These new sources show similar tolerance to the previously identified donors commonly used Pokkali group and Nona Bokra. These landraces are currently being cultivated by farmers in the salt-affected areas of the coastal region of South Bangladesh and West Bengal, India despite their poor grain quality and lower yield. They could also be useful for varietal improvement with conventional breeding tools and new QTLs/genes sources for molecular breeding.

In the present study, screened 3,195 rice germplasms for salinity tolerance, and most of the materials were collected from the BRRI germplasm center. In each group of germplasms sources, the majority of the materials were found sensitive to salinity stress. Rice has been grouped as a salt-sensitive cereal, especially at its early stage (Lutts *et al.*, 1995). From a total of 2,295 germplasm materials, only 120

rice genotypes scored (SES) 3 to 5 based on performance against salinity stress in greenhouse conditions with very few tolerant genotypes. In addition to characterizing physiological responses to salt stress, advances have been made in identifying quantitative trait loci (QTLs) and genes controlling salinity tolerance traits. For example, several QTLs for salt tolerance have been identified in rice, including a major locus on chromosome 1, comprising the major locus *Saltol* obtained from Pokkali (Bonilla *et al.*, 2002) and *SKC1 (OsHKT1;5)* from Nona Bokra. The *Saltol* locus is involved in Na/K homeostasis under salt stress (Platten *et al.*, 2013). These QTLs improve the salinity tolerance of modern high-yielding varieties (HYV) but do not provide complete tolerance in modern HYV background. Thomson *et al.* (2010) conducted experiment to identify and combine genes and QTLs controlling different physiological mechanisms at both the seedling and reproductive stages to rapidly develop rice varieties that can produce higher and more stable yields under high salt stress conditions.

Further, identified other salinity tolerant genes from different sources. By pyramiding, these genes may provide more salinity tolerance in HYV background. It is reported that, seven landraces viz., Akundi, Ashfal, Capsule, Chikirampatnai, JataiBalam, Kalarata and Kutipatnai had accumulated less Na and comparatively more K, retaining a lower Na/K ratio in the leaves (Rahman *et al.*, 2016). They essentially restrict the transport of sodium to the shoot.

Along with rice germplasm screening, also screened advanced breeding lines developed in salinity tolerance breeding programs both from BIRRI and IRRI. To develop high-yielding salt-tolerant varieties, screened a total of 193 BIRRI lines developed at BIRRI. Only 41 advanced breeding lines scored (SES) 3 to 5 based on screening results against salinity stress in greenhouse conditions. Among them, most of them were sister lines and originated from the same cross. Among tested BIRRI lines, some good breeding lines were promoted as salt-tolerant rice varieties. Based on better performance and higher grain yield in actual salinity areas, the advanced breeding lines BR5999-82-3-2-HR1, BR7105-4R-2, and BR7100-R-6-6 were released as BIRRI dhan54, BIRRI dhan61, and BIRRI dhan67, respectively from BIRRI. BIRRI dhan67 can tolerate 8 dS m⁻¹ salinity (BIRRI, 2018) with grain yield of 6.2 t ha⁻¹ yield at south Bangladesh under high salinity regime. BIRRI researchers has developed a high-yielding salt-tolerant variety CSR23 (also called as IET13769), which can tolerate pH 2.0±10.0 and salinity up to 8 dS m⁻¹ (Singh *et al.*, 2006). CSR23 is better in K⁺ uptake and Na⁺ exclusion and suitable for the majority of coastal regions.

Like BIRRI lines, IRRI lines also showed relatively more potentiality with a better performance against salinity stress. One hundred and eighty-one advanced breeding lines SES score ranked 3 to 5 based on screening results against salinity stress in greenhouse conditions from a total of 707 IRRI lines produced at IRRI. Recently, a salt-tolerant transgenic line from IR64 variety with overexpressing PcINO1 from halophytic wild rice called *Porteresia coarctata* has been developed. *P. coarctata* is a landrace in India, Sri Lanka, Bangladesh and Myanmar. Transgenic rice tolerates upto 200 mmolL⁻¹ salt or higher concentration in pots by inositol production under saline conditions and shows normal growth and grain yield under greenhouse conditions (Mukherjee *et al.*, 2019). Among the promising IRRI developed breeding lines, IR63307-4B-4-3 and IR78761-B-SATB1-28-3-26 were released as BIRRI dhan47 and BIRRI dhan73. The ideal salinity tolerant variety should possess tolerance to the high amount of Na⁺, control the uptake of Na⁺ and keep high uptake of K⁺, good initial vigor, agronomically superior with high yield potential.

Before the yield trial, these breeding lines were subjected to screening against salinity in greenhouse conditions and they exhibited a score (SES) 3 during the screening period. These results suggested that the screening results effectively identify promising materials from a large set of accessions. Salinity tolerance is a complex trait in rice and controlled by polygenes. As a whole, the identified tolerant rice genotypes from three different sources might have potentiality in developing advanced breeding lines with improved salinity tolerance in rice. Pokkali and Nona-bokra and their derived lines have been widely used as saline tolerant donors in developing saline tolerant rice varieties and identifying candidate genes against salinity (Rahman *et al.*, 2016). Recent advances in genomics also suggested the

use of diverse parents in developing elite lines from germplasm. Moreover, this study's identified materials may serve as a key founder in producing breeding lines with improved salinity tolerance.

Conclusion

Screening and identification of new tolerant breeding lines is a continuous work. In the present study, extensive scale screening of rice seedlings was performed against major abiotic stress, salinity to identify promising genetic materials and sources. However, much effort is required to develop tolerance breeding lines across the whole life cycle specially in reproductive stage. In the future, promising materials from the identified set may be tested in both the seedling and reproductive stages.

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