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Research Article

Phenotypic Parameters Clustering Based Screening of Rice (*Oryza sativa* L.) Landraces for Salt Tolerance

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Abstract

Background and Objectives: Center of origin possesses the largest diversity of any germplasm. Bangladesh is a reservoir of diverse rice germplasm with unique and important traits. Characterization of these landraces can suggest how they survive in saline soils and their potential use in breeding programs. Therefore, this experiment was conducted to find out potential salt tolerant rice landraces. **Materials and Methods:** Twenty one rice genotypes were selected and among them salt tolerant genotypes were screened out at seedling stage based on phenotypic parameters. Rice seedlings were grown in the hydroponic nutrient medium in 0 and 8 dSm⁻¹ conditions in completely randomized design with three replications. Calculations and data analyses were performed using the Minitab 17. **Results:** Salinity stress reduced phenotypic parameters and also reduced stress tolerance index (STI) for different parameters. The maximum stress tolerance index value for shoot length, root length and total dry weight were noticed in BINA dhan-8, Bolonga and BINA dhan-10, respectively. Compiling all phenotypic data in Pearson absolute correlation coefficient distance based cluster analysis, Gajor Goria, Bolonga, Bina sail and Nakraji were identified as highly salt tolerant and Dud Sail, Tal Mugur, Gota, Sona Anjul, Kolmilota, Konkacur and Panbra were identified as moderately salt tolerant rice landraces. **Conclusion:** Gajor Goria, Bolonga, Bina sail and Nakraji can be used for the breeding of high yielding salt tolerant rice varieties.

Key words: Landraces, salt stress, morphological parameters, phenotypic screening, hydroponic culture, seedling stage

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Salinity is an ever-present threat to the productivity of crops including rice throughout the world. A soil can be termed as saline if its EC is 4 dSm^{-1} or more¹. Salt stress is known to cause osmotic stress, ionic toxicity, oxidative stress and nutritional imbalance in plants². The physiological toxic effects of salt stress include decreased germination and seedling growth, reduced leaf expansion which causes a reduction in the photosynthetic area and dry matter production³. Globally salt affected area accounts to about 1 billion hectares of land⁴. The saline area is three times larger than land used for agriculture⁵. In Bangladesh, coastal area has already been experiencing erosion. It has been found that the sea level rise of 0.5 m over the last 100 years has eroded approximately 426 km² of different coastal areas of Bangladesh⁶. If the trend continues, sea water may intrude much longer distance in inland extending towards the interior coast in low-lying areas of Bangladesh⁷. It extends upto 150 km from the coast. Out of 2.85 million hectares of the coastal and offshore areas about 0.83 million hectares are arable lands, which cover over 30% of the total cultivable lands⁸.

Rice (*Oryza* spp.) is an important cereal crop and is mainly used for human consumption. It's a staple food and cash crop for more than three billion people in the world⁹. Rice covers a global area of 156 million hectares of land producing about 650 million tonnes of crops¹⁰. Asian farmers constitute about 92% of the world's total rice producing group¹¹. Rice has been feeding the region's population for well over 4000 years and is the staple food of about 557 million people¹². In Bangladesh, rice provides nearly 48% of rural employment, about two-third of total calorie supply and about one-half of the total protein intakes of an average person in the country and rice sector contributes one-half of the agricultural GDP and one-sixth of the national income¹³.

Rice is relatively more sensitive to salinity than other cereal crops such as barley and wheat¹⁴. Rice production systems of the region have over recent years become increasingly threatened by the effects of salinity¹⁵, as a large portion of the rice growing areas is located in especially vulnerable regions. Development of salt tolerant varieties is the most cost effective and reasonable approach to mitigate the salinity stress. For this, the foremost step is to screen the existing germplasm of paddy to identify the potential breeding materials. Rice is genetically diverse for having many landraces and progenitor species. It is a highly polymorphic crop species with wide geographic distribution¹⁶. Moreover, traditional landraces are important reservoirs of valuable trait and need attention for conservation and improvement¹⁷.

Landraces are widespread and popular among farmers and are an important part of agriculture because their diverse array in a crop creates genetic diversity in agriculture¹⁸. Landraces are known to be heterogeneous mixtures of genotypes carrying a range of stress tolerance genes¹⁹. Bangladesh is endowed with a great diversity of rice landraces in its vast traditional land area. After the green revolution, the traditional rice landraces were eliminated majorly by high yielding varieties. Special quality rice landraces have great importance in abiotic stress tolerance related study. Landraces of rice played a very important role in the local food security and sustainable development of agriculture, in addition to their significance as a genetic resource for rice genetic improvement²⁰. Landraces provided "adaptability genes" for specific environmental conditions. Incorporation of adaptability genes from landraces could ensure optimum grain yield for the region.

Variability in salinity tolerance has been reported in rice at different stages of growth, with germination and active tillering stages being more tolerant than panicle initiation, fertilization and early seedling stages²¹. Screening of germplasm at seedling stage is readily accepted as it is based on a simple criterion of selection, it provides rapid screening, which is difficult at the vegetative and reproductive stage²². Screening under controlled condition has the benefit of reduced environmental effects and the hydroponic system is free of difficulties associated with soil-related stress factors. Mass screening and physiological characterization of rice genotypes may help in tailoring salt resistant rice genotypes. This study was conducted to screen different rice landrace genotypes under salt stress on the morphological basis to find out potential salt tolerant rice genotypes.

MATERIALS AND METHODS

Plant materials, plant growing and salinity stress: This experiment was carried out at the laboratory of the Department of Biochemistry and Molecular Biology, Bangladesh Agricultural University and Biotechnology Division, Bangladesh Institute of Nuclear Agriculture, during the period March, 2015-June, 2015.

Twenty-one rice (*Oryza sativa* L.) genotypes, including 15 landrace rice genotypes and four rice cultivars kindly provided by the Biotechnology Division, Bangladesh Institute of Nuclear Agriculture, Mymensingh and two high yielding rice cultivars obtained from Bangladesh Rice Research Institute, Gazipur were screened for their salt tolerance levels at the seedling stage.

BINA dhan-8, BINA dhan-10, FL-478 and BRR1 dhan 53 salt tolerant rice genotypes and the salt susceptible BINA dhan-7 and BRR1 dhan 29 were used as a standard check in salt tolerance screening.

For an establishment of seedlings, rice seeds were surface sterilized, soaked in distilled water for 24 h and then germinated on wet filter papers embedded in petri dishes. Subsequently, 3 days-old seedlings of each rice genotypes raised in petri dishes were transplanted into styrofoam seedling float (28×32×1.25 cm containing 100 (10×10) holes with nylon net at the bottom) fitted in a rectangular glass fibre tray with 12 L capacity and 14×30×35 cm size. The tray having tap water and germinated seeds allowed to grow 3-4 days. After 4 days when the seedlings were well established then the water was replaced with a salinized nutrient solution (Peters water soluble fertilizer (Urea:TSP:MP = 20:20:20) and ferrous sulphate heptahydrate (FeSO₄·7H₂O)) for salinized setup and for control setup only nutrient solution was used. Seedlings of each rice genotype were subjected to salinity stress at 8 dSm⁻¹ for 18 days. Completely randomized design (CRD) with three replication was used in this experiment.

Sampling was performed at the end of the experiments and morphological changes were evaluated. Scoring of visual salt stress injury and growth reduction of rice seedlings treated with 8 dSm⁻¹ salinity was performed using the Standard Evaluation System of rice²². Seedling shoots and roots were separated into an aerial and below-ground part for shoot length and root length determinations, as well as the total dry weight (TDW), was determined after oven drying at 60°C for 3 days. The stress tolerance index (STI) was calculated using the formula according to Zeng *et al.*²³:

$$\text{Stress tolerance index (STI)} = \frac{\text{Observed value of a trait under salinity level}}{\text{Mean value for that trait under the control}} \times 100$$

Statistical analysis: All calculations and data analyses were performed using the Minitab 17 for windows software package. All the data obtained were converted to salt tolerance indexes before Pearson's correlation and cluster analyses. Pearson absolute correlation coefficient distance based cluster analysis was also performed using the Minitab 17 package.

RESULTS

Shoot length STI under salinity stress: Rice seedlings grown in salinized condition showed a decrease in the shoot length of plants than non-saline conditions. Some genotypes showed better shoot growth than other genotypes (Fig. 1a) which may be due to their ability to tolerate salt stress. The STI for shoot length at 8 dSm⁻¹ salt stress ranged from 49-80. The maximum STI value for shoot length was noticed in BINA dhan-8 closely followed by BINA dhan-10, FL-478, Bina sail and Nakraji, respectively. On the other hand, the minimum STI for shoot length was marked in Dud sail.

Root length STI under salinity stress: The STI for root length ranged between 99% for BINA dhan-8 and 71% for BINA dhan-7 (Fig. 1b). Some landraces showed similar STI for root length to the tolerant check varieties and some showed similar to susceptible check varieties. Genotypes with higher STI were considered as tolerant and with lower STI considered as susceptible. Based on this, the tolerant genotypes are Bolonga, Gota, Dud sail and Nakraji.

Total dry weight STI under salinity stress: The STI for total dry weight at 12 dSm⁻¹ saline condition ranged between 29-83%. Total dry weight STI was with the highest value at salinity in genotypes BINA dhan-10 followed by FL-478, BINA dhan-8 and Bolonga, while it was noted minimum in Sona Anjul followed by Konkacur and Tal Mugar (Fig. 1c).

Cluster analysis: Fifteen rice landraces, BINA dhan-7, BINA dhan-8, BINA dhan-10, FL-478, BRR1 dhan 53 and BRR1 dhan 29 were analysed for physiological responses based on salinity tolerances at the young seedling stage. The results showed that all varieties were classified into three main groups including tolerant, moderately tolerant and susceptible (Fig. 2). It was found that out of 15 landraces, 4 were tolerant, 7 were moderately tolerant and 4 were susceptible. BINA dhan-8, BINA dhan-10, FL-478, Gajor Gorja, Bolonga, Bina sail and Nakraji were classified in the salt tolerant group while BINA dhan-7, BRR1 dhan 29, Sona Toly, Tilkapur, Beto and Patnai Balam were in the susceptible group. The salt tolerant rice exhibited some important characteristics such as a higher survival rate and a lower salt injury score than the sensitive plants. In addition, Dud Sail, Tal Mugar, Gota, Sona Anjul, Kolmilota, Konkacur and Panbra were identified as moderately tolerant landraces in this study.

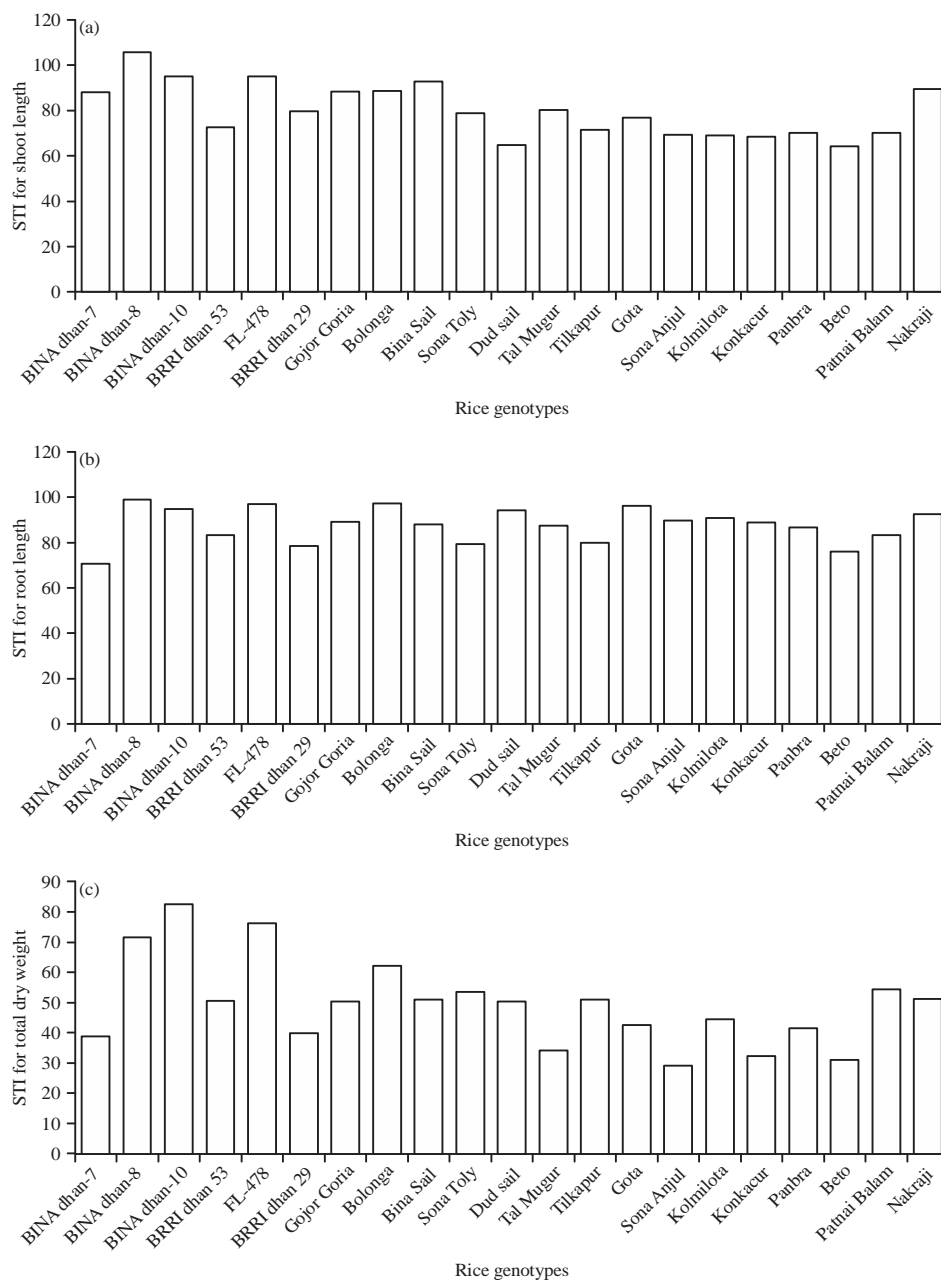


Fig. 1(a-c): Effect of salinity on (a) STI for shoot length, (b) STI for root length and (c) STI for plant dry weight at 18 days after exposure to saline nutrient solution for 21 rice genotypes

DISCUSSION

Finding out potential rice genotypes is critical to current and future breeding efforts to improve rice yield in the areas affected by soil salinity. Considerable efforts have been made so far to identify salt tolerant rice genotypes but still now some landraces were not evaluated for salinity tolerance.

The shoot length, root length and plant biomass in different genotypes under study showed wide fluctuations.

Rice is very sensitive to salinity at different growth stages, especially at seedling stage. The growth of crop plants is adversely affected by salt stress because of limited absorption of water through roots. Salinity stress imposes an immediate effect on cell growth and enlargement and high concentration of salts can be extremely toxic¹⁴.

In the present study, salt stress remarkably reduced various growth attributes such as shoot length, root length and total dry matter of rice genotypes studied. As a result, the

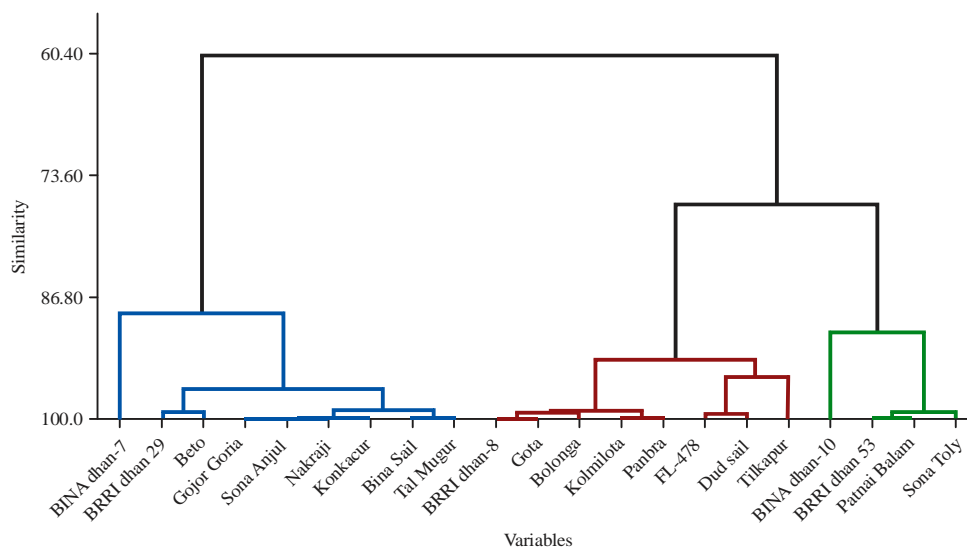


Fig. 2: Cluster of 21 genotypes based on different phenotypic parameters under study using Pearson's absolute correlation coefficient distance

STI for different parameters are also being adversely affected by salinity stress (Fig. 1). However, the extent of STI reduction under salt stress was dependent on genotypes. Salt sensitive genotypes showed more reduction in their STI as compared to tolerant genotypes. The STI for shoot length was reduced with the increase of salinity. Considering the extent of STI for shoot length, the genotypes BINA dhan-8, BINA dhan-10, FL-478 and Nakraji showed the highest value (Fig. 1a). The STI for root length was also decreased with an increase in salt stress. Considering the STI for root length the genotypes BINA dhan-8, BINA dhan-10, FL-478, Bolonga and Nakraji appeared superior (Fig. 1b). These genotypes had the ability to maintain their root length reasonably well even in the saline growth medium. Djanaguiraman *et al.*²⁴ reported a significant decrease in root and shoot lengths with increase in salt concentration. Roots have direct contact with soil for water and minerals uptake, so root characters can effectively be used as selection criteria in breeding for salinity tolerance.

In this study, STI for shoot length was highly affected by salinity than STI for root length (Fig. 1b). This might be due to the reason that plants, especially those of drought or salt tolerant species, tend to propagate their roots deeper to absorb more water during osmotic stress. These results are in agreement with the findings of Haq *et al.*²⁵, who reported the differential response of rice genotypes under salinity stress.

In our investigation, total dry biomass showed a greater reduction in sensitive genotypes than tolerant genotypes. Under salinity stress, STI for the total dry weight of plant was considerably reduced. The genotypes BINA dhan-8, BINA dhan-10 and FL-478 could retain their biomass efficiently

under salinity condition (Fig. 1c). An increase in plant height during stress results in an increase in plant's biomass. Dry biomass, especially at the seedling stage has been found associated with salt tolerance in crop plants and can therefore, be used as an indicator of salt tolerance or sensitivity. The findings in this study that the rice plants exhibited a significant reduction in their dry weight were consistent with Amirjani⁵, who reported that salt tolerance levels decreased as the NaCl concentration increased in rice cv. Tarom Azmoon at the seedling stage. Tatar *et al.*²⁶ also reported salt stress significantly reduced the total dry matter of rice cultivars at the seedling stage. Likewise, Senadheera *et al.*²⁷ observed that salt stress of 50 mM NaCl caused a significant decrease in both fresh weight and dry weight of the salt sensitive IR29 at the seedling stage. Bhowmik *et al.*²⁸ reported that plant height and total dry matter of tolerant lines of rice were reduced by 19.0 and 40.6%, respectively under salt stress (EC 12 dSm⁻¹), whereas, those of susceptible lines were reduced by 46.0 and 73.5%, respectively.

This study results showed a reduction in shoot and root growth of rice genotypes under salinity stress that resulted in reduced shoot and root lengths as well as STI (Fig. 1). The sensitive genotypes exhibited various symptoms of salt injury such as yellowing of leaf, reduction in root and shoot growth and ultimately dying of seedlings at the vegetative growth stage. Mansuri *et al.*²⁹ evaluated 15 rice genotypes for salt tolerance and reported growth reduction, rolling and drying of leaves and reduction in seedling height under saline conditions. They also found reduced root/shoot dry weight under salinity stress and reported higher biomass in tolerant

genotypes compared to sensitive ones. Among the 15 landraces, the genotypes showed similar STI compared to tolerant check varieties were considered as tolerant ones. They concluded that biomass was positively correlated with salt stress tolerance and, therefore, can be used as a selection criterion for salt tolerance²⁹.

The genotypes closest to each other are grouped into one cluster. Gajor Goria, Bolonga, Bina sail and Nakraji were classified in the salt tolerant landraces in the cluster analysis (Fig. 2). Different researchers have used cluster analysis to group different crop genotypes based on various characteristics and found similarities of crop genotypes within a group³⁰. The literature also emphasizes the use of cluster analysis to screen the crop germplasm for stress tolerance³¹⁻³³.

CONCLUSION

Gajor Goria, Bolonga, Bina sail and Nakraji performed as a highly tolerant under the saline condition and its tolerance to salinity. So this genotype could be used as a potential donor of saltol gene. In this regards further study is recommended. In addition, Dud Sail, Tal Mugur, Gota, Sona Anjul, Kolmilota, Konkacur and Panbra were identified as moderately tolerant landraces which also can be used for the development of new salt tolerant varieties. This study only focused at seedling stage and further study is recommended at reproductive stage.

SIGNIFICANCE STATEMENTS

This study discovers some salt tolerant rice landraces that can be beneficial for plant breeders and biotechnologists. These salt tolerant rice genotypes can be used for the development of high yielding salt tolerant rice varieties. This study will help the researcher to uncover the critical areas of plant salinity stress tolerance in some rare indigenous landraces that many researchers were not able to explore.

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