COLLECTION AND SCREENING FOR SALINITY STRESS TOLERANT RICE GENOTYPES USING COMBINATION OF MORPHOPHYSIOLOGICAL AND MOLECULAR MARKER

Dr. Jamilur Rahman[1]

Extended Summary

Rice is the main food crop and it provides up to 80% of the daily energy intake in Bangladesh. However, the rate of increase of rice production is slowing down and if the trend is not reversed, severe food shortage will be occur in near future. To meet the food demand in future, it is necessary to increase rice production either by increasing the yield per unit area and/or by increasing the rice cultivated land areas. In Bangladesh about 1 million hectares of costal land are affected by salinity. There are widespread soil problems and salinity that impair normal growth and limit the realization of yield potential of rice varieties in the saline areas. Therefore, the best possible way to increase the rice production by utilizing the coastal saline affected lands is to develop salinity tolerant modern rice varieties.

Salinity affects almost every aspect of the physiology and biochemistry of plants and significantly reduces yield. As saline soils and saline waters are common around the world, great effort has been devoted to understanding physiological aspects of tolerance to salinity in plants, as a basis for plant breeders to develop salinity-tolerant genotypes. Salt tolerance is a polygenic character. Exploitation of germplasm is one the best probable ways to breed salinity tolerant crop varieties. Characterization of the germplasms to indentify the promising rice genotypes of saline tolerance is one of the most important research activities for further improvement of saline tolerance rice varieties. The germplasms is the main reservoir and they may contain novel genes and QTLs and adapt novel salt tolerance mechanism to survive in saline stress condition. Therefore, characterization of these germplasm may unravel novel salinity tolerant mechanism and novel genes, which will definitely assist to breed/improve efficient and modern salt tolerance rice varieties. Characterization of diverse genetic resources for traits of interest is an essential part of commencement of breeding program. Predominantly, genetic improvement in mainly depends on the amount of characterized genetic variability present in the population. Hence, characterization and estimation of genetic variability or diversity for salt tolerance parameters existing in the pool of rice germplasms is imperative in planning for future breeding program. Therefore, in the present project an attempt was taken to characterize the diverse rice germplasms. Germplasm is the main reservoirs of many known and unknown novel genes and QTLs as well as the main capital for the breeders to improve and breed the

[1] Associate Professor, Dept. of Genetics and Plant Breeding, Sher-e-Bangla Agricultural University, Dhaka- 1207

new varieties. To enrich the pool of diverse genetic materials for salinity tolerance trait we collected more than 45 diverse rice germaplasms from south-eastern districts of Bangladesh and research institutes, e.g. BINA and BRRI (Table 1).

Table 1. Rice germplasms collected from south-eastern part and different research institutes of Bangladesh

1.	Kajol Shai	16.	THDB	31.	Mut -1-1
2.	Patani	17.	Koakhata	32.	Charnock
3.	Montasor	18.	BINA Dhan – 8	33.	Mut -1-1
4.	Meshi Jabra	19.	IR-24	34.	Dholiboro 105/2
5.	Nahra Jamai Nar	20.	Bora Dhan	35.	PNR-519
6.	Mohini Solt	21.	FL-478	36.	FL - 378
7.	Sada Gotal	22.	Janglai Boro	37.	Dhud Kalam
8.	Jamai Naru	23.	BINA Phul R6-59	38.	Kali Boro – 109/4
9.	Gossa Aman	24.	Y-1281	39.	Kali Boro – 138/2
10.	Rani Salt	25.	Patnai (FW)	40.	PBSAL 655
11.	Kumra Gour	26.	Chini Kani	41.	RD- 2586
12.	Sada	27.	Bawoi Jakh	42.	Ketrarail
13	Pokkali	28.	PBSAL 730	43.	PBRC-30
14	BRRI Dhan -40	29.	STL-15	44.	Kherri
15	BRRI Dhan -47	30.	Dhol Kuchuri	45.	IR 64

The viability of the collected 45 germplasms was tested through seed germination test in the petri dishes. Fifteen rice seeds from each 45 lines were soaked in test tubes containing autoclaved distilled water for 24 hrs. in dark condition at 30°C in incubator. The soaked rice seed are then placed in the petri dish containing soaked whatman sheet (3mm). Viable rice seeds were found germinated within 4-8 days. We found 50% of the collected rice germplasms were germinated with 3-5 days and 40% germinated within 6-8 days and rest 10% were not shown germinated at all. The differential germination period observed in the diverse germpalsms might be attributed to differential inherent dormancy period of different rice genotypes.

For proper screening of salinity tolerance rice germaplasms, a uniform seed germination is prerequisite because delay in germination of some entries may likely make these entries more sensitive to salt. Proper breaking of the seed dormancy is essential in thus screening technique. Therefore, we broke the seed dormancy to make uniform germination. For breaking the seed dormancy the test rice seeds were heat-treated for 5 days in a convection oven set at 50°C. After breaking the dormancy we found all entries were germinated uniformly (i.e. within 3-5 days). Rice seeds were surface sterilized with 75% ethanol for 3 min, 15% (v/v) sodium hypochlorite solution containing Tween-20 as wetting agent for 15 min. The seeds were then rinsed four times with sterile autoclaved distilled water and then imbibed in water for 48 hrs at room temperature in dark condition.

The screening of salt tolerance rice genotype was performed following the screening technique suggested by IRRI. The surface sterilized rice seeds were floated on the Styrofoam floats. A float is a fabrication of a rectangular Styrofoam having holes with nylon net bottom. The float was kept in a rectangular commercial plastic tray (approx. size: 12 x 6 x 3 inches^3). The pre-socked and surfaced sterilized rice seeds were kept in the holes of Styrofoam floats. For salinization, Yoshida nutrient liquid medium (Yoshida et al., 1976) containing different concentration (0, 6, 12, 16 dSm^{-1}) of NaCl was poured in the plastic tray. We salinized the nutrient solution by adding NaCl while stirring up to the desired EC (e.g. 6 and 12 g NaCl per Liter nutrient solution gives an EC of 6 and 12 dS m^{-1} respectively). The plastic tray was filled up with this solution high enough to touch the nylon net bottom of the Styrofoam. The effective culture solution needed per tray is about 2-3 L per plastic tray. For germination of the seeds and grown up of the rice seedlings, we used a culture chamber (Labtech Co. Ltd.). The culture environment included 31°C temperature, 75.0% relative humidity and a 16-h photoperiod from white fluorescent lamps (300 μmol photons/m^2/s^{-1}). We carefully maintained the pH of the Yoshida nutrient saline solution because significant deviation (±1.0) of culture solution pH from 5.0 would make some nutrients toxic and others deficient. The saline liquid nutrient medium was changed in every three alternative days to avoid the contaminations. For evaluation of the salinity tolerance rice genotype, we used modified standard evaluating score (Table 2) in rating the visual symptoms of salt toxicity.

Table 2. Modified standard evaluation score of visual salt injury at seedling stage

Score	Observation	Degree of 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

The seedling float had 6 rows with 9 holes each. One row was used for one test entry. It is essential to have check varieties in every seedling float to guide in rating the visual symptoms of salinity stress. We used three check varieties BRRI Dhan 28, sensitive; BRRI Dhan 47, moderately tolerant (both improved varieties from BRRI); and Pokkali, tolerant (traditional variety). This scoring discriminates the susceptible from the tolerant and the moderately tolerant genotypes. Initially, we found ten promising salt tolerant rice genotype, however, for further validation the experiment needs to be conducted in field condition.