



Evaluation of the genetic variability and genetic advance for salt tolerance in rice genotypes

MM Huda, A Ferdousi, L Hassan, MA Hossain*

Department of Genetics and Plant Breeding, Faculty of Agriculture, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh

Abstract

Soil salinity is considered as one of the greatest threats for rice production. Genetic variability analysis of morphological traits related to salt injury is mandatory, which could be helpful in the development of an appropriate breeding program for the selection of salt tolerant rice genotypes from the existing germplasm towards developing new salt tolerant rice varieties. Therefore, the present study has been aimed to evaluate the genetic variability parameters for fourteen morphological traits in thirty local and high yielding rice genotypes using hydroponic culture. Among the rice genotypes under study, Boilam, Nonabokri, Gunshi, Motabamonkhir showed the best performance regarding the morphological traits under different saline conditions (6 dSm^{-1} and 10 dS m^{-1}). The PCV values were greater than the GCV values for all studied traits indicating the greater environmental influence on the traits. The highest percentage of GCV and PCV were observed in root dry weight, while the lowest were exhibited in main axis diameter. To assist the breeding program, along with variability, estimation of heritability and genetic advance has also been done. High heritability ranging from 80.24% to 94.66% was found among the traits. The genetic advance was highest for survival rate and the lowest was in root dry weight. Eventually, the results indicated the presence of high genetic variability, along with high heritability and genetic advance among genotypes and studied traits, which could provide further insight towards selection of parents for the development of salt tolerant rice varieties.

Key words: Rice, genetic variability, heritability, genetic advance, salt tolerance

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*Corresponding Author: amir04@gmail.com

Introduction

Rice (*Oryza saliva* L.) is one of the most important cereals in the world ensuring world food security as it is the staple food near about 50% of the total worlds' population with the third-highest worldwide production, after sugarcane and maize (FAOSTAT, 2015). Among the 95 rice producing countries in the world, Bangladesh holds the fourth position in terms of total rice production (FAO, 2014) of about 34,500 thousand metric ton cultivating about 11,790 thousand hectare of land (USDA, 2015). An increased rice

production was reported through the Green Revolution in 1970s, but afterwards the production rate became slow and steady (Maclean *et al.*, 2002).

Many factors are considered for the low production rate in rice, especially the environmental obstacles such as different abiotic stress e.g. drought, flood and salinity (Gregorio *et al.*, 2002). Among these, soil salinity become more alarming as the amount of saline area around the globe is increasing gradually due to

higher use of ground water for irrigation and rising of the sea level due to the global warming (Mori and Kinoshita, 1987). Hence, it is considered the second most widespread soil problem in rice growing areas of the world after drought (Gregorio *et al.*, 1997). Rice plants are very sensitive to salinity at seedling stage (Akber and Yabuno, 1974), salinity at reproductive stage causes delayed flowering, reduction in the number of effective tillers, the number of grains per panicle, grain weight and ultimately reduce the grain yield (Khatun *et al.*, 1995; Gregorio *et al.*, 2002). Therefore, developing salt tolerant rice varieties have been considered as the most effective strategies to increase rice production in saline areas of Bangladesh bringing the saline areas under rice cultivation.

The improvement of a crop is mostly dependant on the existing genetic variability and heritability (Yared and Misteru, 2016). However, it is estimated that more than 75% of the potential genetic variability and diversity has been unutilized (Nuruzzaman *et al.*, 2017). Therefore, screening of those existing lines with distinct genetic structure might be a good promise for the rice yield improvement (Nuruzzaman *et al.*, 2017). The nature and degree of the variation available in a particular crop and understanding the relationship of environmental influence on the expression of different yield contributing traits are mandatory for any crop improvement program (Bhuvaneshwari, 2008). The success of the crop improvement program depends on various genetic variability parameters along with heritability estimates that could provide the better understanding of genetic advance, which provides precise information on selection (Singh *et al.*, 2017; Parmar *et al.*, 2013). Therefore, the present investigation was undertaken to estimate and evaluate the extent of genetic variability, heritability and genetic advance for different characters under saline condition in thirty rice genotypes for the screening of saline tolerant genotypes.

Materials and Methods

Plant materials: The hydroponic experiment and morphological analyses were conducted in the growth chamber of the Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh, Bangladesh. Thirty rice genotypes (Table 1) were evaluated for their salt tolerance ability, among which twenty-four varieties were local landraces and six were high yielding varieties (HYVs).

Table 1. List of rice genotypes with their types and source of collection

Types	Genotypes	Source
Local Varieties	Chapsali, Jamainadu, Jataibalam, Horkuch, Jamaibabu, Ghigoj, Hogla, HonumanJata, Changai, Motabamonkhir, Birpala, Bashfulbalam	Thikeirum, Dorkumor, Kutipathai, Chiknul, Boilam, Gopalbhog, Nonabokri, Chinikanai, Ashfal, Gunshi, Pokkali
HYVs	BRRRI dhan 61, BRRRI dhan 41, BRRRI dhan 23, BRRRI dhan 40, BRRRI dhan 54, BRRRI dhan 55	BRRRI

BRRRI = Bangladesh Rice Research Institute, Gazipur, Bangladesh

Hydroponic culture: The hydroponic experiment was conducted in a well facilitate growth chamber of a concreted room (10×11) square feet with controlled light (white fluorescent lamps: 240 lumens and compact fluorescent lamps: 1890 lumens) and temperature (window air conditioner: Measuring range: -10°C to 110°C) facilities.

Seeds were placed into brown bags and were kept in oven (55°C) for 2 to 3 days to break the seed dormancy. Seed sterilization was performed using

0.1% HgCl₂ and 70% ethanol and then washed several times with distilled water. Sterilized seeds were placed in petridishes, containing moist filter paper and kept in the dark for 3 to 4 days for sprouting. The 4 to 5 days old seedlings were then wrapped in sponge and transferred to the hydroponic medium (Peters Professional® Product User Guide, ICL Fertilizers Co.). Two salt treatments (6 dSm⁻¹ and 10 dSm⁻¹) were used by adding 2g and 4.25g NaCl to the normal nutrient solution (1.3 dSm⁻¹), respectively to observe the performances of rice seedlings at different salinity levels. Seedlings were treated to salt treatments for seven days and then they were transferred to the normal nutrient solution.

The IRRI (International Rice Research Institute) prescribed modified SES (Standard Evaluation Score) was used to estimate the visual symptoms of salt toxicity (Table 2). The scoring was used to discriminate the genotypes into tolerant, moderately tolerant, and susceptible. The scoring was done after 14 to 16 days after salt treatment.

Table 2. Modified Standard Evaluation Score (SES) for visual salt injury (score scale 1 to 9) (IRRI, 2010)

Score	Observation	Tolerance level
1	Normal growth, no leaf symptoms	Highly tolerant
3	Nearly normal growth, but leaf tips of few leaves whitish and rolled	Tolerant
5	Growth severely retarded , most leaves rolled; only a few are elongating	Moderately tolerant
7	Complete cessations of growth, most leaves dry; some plants dying	Susceptible
9	Almost all plants dead or dying	Highly susceptible

Data collection and analysis: Data were collected on different shoot (percent of live leaves, main axis diameter, number of primary axis, primary axis diameter, primary axis length and shoot dry weight) and root (root length, total number of roots and root dry weight) parameters after 18 days of hydroponic culture by successive destructive harvest. The experiment was designed followed by two-factor Randomized Complete Block Design (RCBD) with three replications. Analysis of variance (ANOVA) was performed using the plant breeding statistical program (Utzal, MSTATC and PLABSTAT, Version 2N, 2007) with the following methods:

$$Y_{ij} = g_i + r_j + e_{ij}$$

Where,

Y_{ij} = Observation of genotype i in replication j

g_i = Effects of genotype i

r_j = Effects of replication j

e_{ij} = The residual error of genotype i in replicate j

Genetic parameters: Genotypic and phenotypic variances were estimated according to the formula given by Johnson *et al.* (1955). Heritability in broad sense (h²_b) was also estimated according to the formula suggested by Johnson *et al.* (1955). Genotypic (GCA) and phenotypic (PCA) coefficient of variations were estimated according to Burton (1952) and Singh and Choudhury (1985). Estimation of genetic advance (GA) was done following the formula given by Johnson *et al.* (1955) and Allard (1960). Genetic advance in per cent (% GA) of mean was calculated by the formula of Comstock and Robinson (1952).

Results and Discussion

Various stress symptoms were recorded under different levels of salinity (control, EC-6 dSm⁻¹ and EC-10 dSm⁻¹) such as yellowing of leaves, drying of leaves, leaf curling, reduced growth rate and seedling drying (Figure 1). Similar stress symptoms were also detected in previous studies (Bonilla *et al.*, 2002; Niones, 2004; Islam, 2004; Bhuiyan *et al.*, 2005). Whereas, the seedlings that were not exposed to salinity showed normal growth and salt tolerant genotypes (according

to SES, Table 4) showed relatively better growth and lower symptoms than susceptible genotypes in saline condition (according to SES, Table 4) (Figure 1).

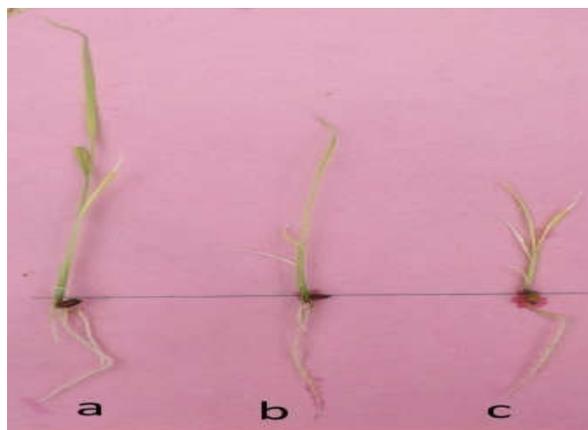


Figure 1. Level of salt injuries at different degrees of salinity (a) control, (b) EC-6 dSm⁻¹, and (c) EC-10 dSm⁻¹

Two rice genotypes were found highly tolerant (without any stress symptoms), eight genotypes as tolerant (minimum level of stress symptoms), fifteen genotypes as moderately tolerant (high level of stress symptoms but survived) and remaining five of them were found susceptible at lower salt treatment (6 dSm⁻¹) (Table 3).

On the contrary, at higher salt concentration (10 dSm⁻¹) most of the genotypes (fifteen out of thirty) were found susceptible. Some of which were moderately tolerant at lower salt concentration (e. g. Dorkumor, BRRI dhan 61, Jataibalam and Kutipathai). Eleven genotypes were moderately tolerant and only four genotypes were tolerant at high salinity (Table 3).

Table 3. Evaluation of thirty rice genotypes at seedling stage under different saline conditions based on Standard Evaluation Score (SES)

Salinity level	Tolerance level (SES scores)	Genotypes
EC-6 dSm ⁻¹	Highly tolerant (2.3 to 2.4)	Nonabokri, Motabamonkhir
	Tolerant (3.4 to 4.7)	Chapsali, Jamainadu, Boilam, Akundi, Hogla, Gunshi, Pokkali, Bashfulbalam
	Moderately tolerant (4.8 to 6.4)	Dorkumor, BRRI dhan 61, Jataibalam, Kutipathai, Horkuch, Chiknul, Gopalbhog, Chinikanai, BRRI dhan 40, HonumanJata, Ashfal, Changai, BRRI dhan 54, Birpala, BRRI dhan-55
	Susceptible (7.0 to 8.5)	Thikeirum, Jamaibabu, Ghigoj, BRRI dhan 41, BRRI dhan 23
EC-10 dSm ⁻¹	Tolerant (4.0 to 4.5)	Boilam, Nonabokri, Gunshi, Motabamonkhir
	Moderately tolerant (5.4 to 6.8)	Chapsali, Jamainadu, Horkuch, Chiknul, Gopalbhog, Hogla, BRRI dhan 40, Changai, BRRI dhan 54, Pokkali, Birpala
	Susceptible (6.9 to 8.6)	Thikeirum, BRRI dhan 61, Dorkumor, Jataibalam, Kutipathai, BRRI dhan 41, Jamaibabu, Ghigoj, Akundi, Chinikanai, HonumanJata, Ashfal, BRRI dhan-55 Bashfulbalam
	Highly susceptible (9.0)	BRRI dhan 23

Table 4. Analysis of variance (ANOVA) for different morphological plant characters of 30 rice genotypes under salt treatments

Sources of variation	df	Traits														
		LL	SR	TRT	RL	MAD	NPA	PAL	PAD	nRHMA	LRHMA	RFW	RDW	SFW	SDW	SES
Replication	2	40.80	12.29	0.043	0.571	0.666	0.664	9.92	0.034	4.50	4.40	1.394	0.001	2.38	0.015	0.316
Genotype (A)	29	2695.84**	3956.38**	4.851**	39.538**	33.813**	9.364**	541.21**	1.568**	341.76**	68.87**	44.204**	0.065**	144.15**	6.397**	4.172**
Treatment (B)	2	44101.54**	45117.70**	76.178**	51.906**	9.138**	39.181**	476.66**	0.271**	7487.50**	2655.78**	177.240**	0.290**	597.85**	14.729**	63.961**
A x B	58	361.13**	769.44**	0.646**	2.694**	2.325**	1.486**	62.66**	0.071**	35.96**	9.42**	6.695**	0.003**	8.43**	0.355**	0.673**
Error	178	14.81	30.80	0.205	0.658	1.078	0.316	12.33	0.044	1.72	1.50	0.740	0.001	1.37	0.016	0.151

** indicates significant at 0.01 probability level, **df** = degrees of freedom, **LL** = live leaves (%), **SR** = survival rate (%), **TRT** = total number of roots, **RL** = root length, **MAD** = main axis diameter, **NPA** = number of primary axis, **PAL** = primary axis length, **PAD** = primary axis diameter, **nRHMA** = number of root hairs on the main axis, **LRHMA** = length of root hair on the main axis, **RFW** = root fresh weight, **RDW** = root dry weight, **SFW** = shoot fresh weight, **SDW** = shoot dry weight, **SES** = standard evaluation score

These findings indicate that the tolerance level of rice genotypes decreased by the increase of salinity level. Similar observations were reported in rice earlier in response to salinity by Zeng and Shanon (2000). Therefore, the four genotypes (Boilam, Nonabokri, Gunshi and Motabamonkhir), which were tolerant to highly tolerant in both saline treatments (6 dSm⁻¹ and 10 dSm⁻¹) can be used for further breeding program to incorporate the salinity tolerance in other rice genotypes.

The analysis of variance (ANOVA) of thirty rice genotypes for quantitative traits under different saline conditions represented in Table 4 indicates that the difference among genotypes for all traits under study and standard evaluation score (SES) for salinity were

highly significant for genotype, treatment and genotype-treatment interaction. Significant variations among different morphological traits such as live leaves (%), survival rate (%), total number of roots, root length, root fresh weight, root dry weight, shoot fresh weight, and shoot dry weight have also been observed in rice in response to salinity (Rahmanzadeh et al., 2008; Shereen et al., 2005).

The genetic parameters i.e. genotypic variances (σ^2g), phenotypic variances (σ^2p), heritability, genotypic coefficient of variation (GCV), phenotypic coefficient variation (PCV), genetic advance (GA) and genetic advance in percentage (% GA) for all traits under study are represented in Table 5.

Table 5. Estimation of genetic parameters for morphological characters related to Standard Evaluation Score (SES) in thirty rice genotypes in response to salinity.

Characters	σ^2p	σ^2g	PCV (%)	GCV (%)	Heritability (%)	GA	GA (%)
LL	300.36	259.41	41.30	38.38	86.37	30.83	73.47
SR	441.31	354.11	34.23	30.66	80.24	34.72	56.57
TRT	0.550	0.467	20.40	18.80	84.89	1.30	35.68
RL	4.43	4.09	25.07	24.10	92.42	4.01	47.73
MAD	3.82	3.50	9.59	9.19	91.66	3.69	18.12
NPA	1.06	0.875	15.81	14.38	82.73	1.75	26.95
PAL	60.82	53.17	21.57	20.16	87.42	14.05	38.84
PAD	0.177	0.166	20.60	19.99	94.15	0.815	39.95
nRHMA	38.07	33.98	20.84	19.69	89.25	11.34	38.32
LRHMA	7.74	6.61	28.92	26.72	85.38	4.89	50.86
RFW	4.95	4.17	30.84	28.29	84.15	3.86	53.46
RDW	0.007	0.007	46.51	45.25	94.66	0.166	90.69
SFW	16.09	15.08	43.83	42.43	93.70	7.74	84.61
SDW	0.712	0.671	46.15	44.82	94.33	1.64	89.68
SES	0.472	0.389	21.98	19.95	82.38	1.17	37.30

σ^2p = phenotypic variance, σ^2g = genotypic variance, PCV = phenotypic coefficient of variation, GCV = genotypic coefficient of variation, GA = genetic advance, LL = live leaves (%), SR = survival rate (%), TRT = total number of roots, RL = root length, MAD = main axis diameter, NPA = number of primary axis, PAL = primary axis length, PAD = primary axis diameter, nRHMA = number of root hairs on the main axis, LRHMA = length of root hair on the main axis, RFW = root fresh weight, RDW = root dry weight, SFW = shoot fresh weight, SDW = shoot dry weight, SES = standard evaluation score.

A wide range of genetic variations was observed in thirty rice genotypes for fifteen morphological traits.

Phenotypic variance as well as phenotypic coefficient of variation (PCV) was higher than

genotypic variance and genotypic coefficient of variation (GCV) for all traits under study thus indicated the influence of environmental factors on these traits (Table 5). Among the all traits root dry weight exhibited high estimates of GCV and PCV (45.25% and 46.51%) followed by shoot dry weight (44.82% and 46.15%), shoot fresh weight (42.43% and 43.83%) and percent live leaves (38.38% and 41.30%). On the other hand, survival rate (34.23% and 30.66%), root fresh weight (28.29% and 30.84%), length of root hairs (26.72% and 28.92%), root length (24.10% and 25.07%), primary axis length (20.16% and 21.57%), number of root hairs (19.69% and 20.84%) and total number of roots (20.84% and 20.40%) showed moderate values of GCV and PCV (Table 5).

Number of primary axis (14.38% and 15.81%) and main axis diameter (9.19% and 9.59%) exhibited low genotypic and phenotypic coefficient of variation in percentage (Table 5). Similar findings for genetic parameters were observed in rice genotypes at both seedling and reproductive stage (Bhadru *et al.*, 2012; Prajapati *et al.*, 2011).

The high heritability estimates is mandatory to determine the effective selection for a particular trait for a particular growth condition or overall crop yield. Heritability can be classified as low (below 30%), medium (30-60 %) and high (above 60%) (Johnson *et al.*, 1955). The traits studied in the present investigation expressed high heritability estimates ranging from 80.24 to 94.66% (Table 5). Among the traits, the highest heritability was recorded in root dry weight (94.66%) and survival rate (80.24%) with the lowest. High heritability values indicate that the traits under study are less influenced by environment in their expression and have greater possibility of genetic improvement through selection methods (Bhadru *et al.*, 2012; Prajapati *et al.*, 2011). Heritability in conjunction with genetic advance would give a more reliable index of selection value. The information on genetic variation, heritability and genetic advance helps to predict the genetic gain that could be obtained in later

generations, if selection is made for improving the particular trait under study (Panse and Sukhatme, 1957). In general, the traits that show high heritability with high genetic advance are controlled by additive gene action. Selection for the traits having high heritability coupled with high genetic advance is likely to accumulate more additive genes leading to further improvement of their performance (Panse and Sukhatme, 1957).

In the present study, genetic advance was highest for survival rate (34.72) followed by percent live leaves (30.83), and lowest for root dry weight (0.166) followed by primary axis diameter (0.815) among the traits (Table 5). The genetic advance as percent of mean was highest in case of root dry weight (90.69%), while lowest recorded by main axis diameter (18.12%) among the traits (Table 5). High heritability along with high genetic advance was noticed for the traits like survival rate, percent live leaves primary axis diameter. Other traits showed high heritability along with moderate or low genetic advance, which can be upgraded by inter-mating superior genotypes of segregating population derived from combination breeding as recommended by Samadia (2005).

Conclusion

The present study revealed the morphological traits related to the salinity based on SES score as well as genetic variation observed in thirty rice genotypes in response to salinity. The results also revealed the presence of high genetic variability in several morphological traits, which are also considered as major yield contributing traits in rice among the thirty rice genotypes in relation to SES score. This information about the genetic variability, heritability and genetic advance would be useful for proper identification and selection of appropriate rice genotypes for further breeding programs related to higher yield and salinity tolerance in rice.

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