ISSN 2518–2021 (Print) 2415–4474 (Electronic)

Fundamental and Applied Agriculture

Journal home page: https://www.f2ffoundation.org/faa/index.php/home

Vol. 8(4), pp. 730 – 738: 2023, doi: https://doi.org/10.5455/faa.169405

PLANT BREEDING | ORIGINAL ARTICLE

Screening of Rice Mutants for Salinity Tolerance and Yield

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ARTICLE INFO

Article history
Received: 09 Oct 2023
Accepted: 22 Dec 2023
Published online: 31 Dec 2023

Keywords
Salt tolerance,
Oryza sativa L.,
Hydroponic condition,
Seedling age,
Rice

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ABSTRACT

Soil salinity decreases rice yield by 20%, affecting global rice production including in Bangladesh. Some advanced mutant lines were developed through mutation breeding to observe the performance against salinity at the seedling stage. Mutant lines that are tolerant to salinity at the seedling stage can be used to develop salt tolerant varieties. The first experiment evaluated the salinity tolerance of 20 rice genotypes grown under hydroponic conditions at the seedling stage using a completely randomized design (CRD). Binadhan-10 was used as a salt-tolerant check variety.Salinity level of 15 dS/m, shoot length (SL) and shoot weight (SW) were reduced by 5-44% and 12-22%, respectively, in tolerant genotypes and by 51-73% and 26-75% in susceptible genotypes compared to the control condition. Root length (RL) had a marginal reduction of 10-27% in tolerant genotypes and 18-38% in susceptible genotypes. Susceptible genotypes showed drastic change in SL, SW, shoot dry weight (SDW) and root dry weight (RDW) compared to the tolerant genotypes. According to the heatmap, growth parameters SL, SW, SDW, and RDW formed cluster I while RL formed cluster II. According to the radar plot clustering, tolerant genotypes were in cluster-I, moderately tolerant genotypes in cluster-II, and susceptible genotypes in cluster-III, respectively. Four lines (L-4-P-1-1, LIRG-4Seg-1(1)-3, L-4-P-1(1), and L-4- P-1-P-2) identified as salt tolerant. The second experiment evaluated the yield performance of these lines under normal field conditions. The mutant line LIRG-4Seg-1(1)-3 exhibited higher yield (7.6 t/ha) with higher number of effective tillers per plant (9.0) compared to the tested mutants and a longer panicle length (28.9 cm) compared to Binadhan-10 (23.5 cm). This line along with other selected mutants can be further screened at the reproductive phase or utilized as breeding material to develop a salt tolerant variety.

1. Introduction

Among environmental stresses, the increasing level of salinity has become a major threat for crop cultivation, especially in low-lying coastal regions (Rahman et al. 2016). It causes extensive hazard in Bangladesh (Clarke et al. 2015) as well as other rice-growing countries of the globe (Dasgupta et al. 2015). One-fifth of parts of Bangladesh are encircled by shoreline areas where approximately one million hectares of arable area are influenced by dissimilar dimensions of salinity (Mukta et al. 2017).

Salinity adversely influences plant growth, establishment and productivity (Munns and Tester, 2008) and reduces the longevity of single leaves because of ample salt deposition that triggers reduced grain yield (Moradi and Ismail, 2007). In the coastal region of Bangladesh salinity has increased by about 26% over the last four decades (Mohammad et al. 2017).

Rice (*Oryza sativa* L.) serves over 50% of the globe's demography (Kordrostami et al. 2017) and is also the leading meal of about 166.3 million inhabitants of Bangladesh (BBS, 2022). Bangladesh is one of the most highly populous countries in the world and the prolongation of arable lands to grow rice to confirm accelerated food production is quite illusive. Coastal regions in the southern part of Bangladesh cannot be utilized properly due to lack of salt-tolerant varieties. So, augmentation of salt-tolerant and superior yielding rice cultivars can be the ideal effort to grow rice in these regions to achieve self-adequacy in food production (Hakim et al., 2014). Hybridization and mutation breeding would be the best possible solution to select tolerant lines against salinity (Kamruzzaman et al., 2017).

Screening of rice genotypes at the seedling stage is an easily adaptable and straightforward procedure (Ali et al. 2014). It has been revealed that salt tolerance at the seedling and reproductive stages are distinct from each

Cite This Article
Khanam S, Al Noor MM, Haque MS. 2023. Screening of Rice Mutants for Salinity Tolerance and Yield. *Fundamental and Applied Agriculture*, 8(4): 730–738. https://doi.org/10.5455/faa.169405

other and are governed by diverged groups of genes, indicating tolerance at the reproductive stage is not thoroughly linked with tolerance at the seedling stage and vice-versa. Besides, at reproductive stage salt tolerance is crucial due to impregnation and seed production appears in this period, resulting in the reproductive stage straight away connected to the grain (Ahmadizadeh et al. 2016). To assess salinity tolerance at the seedling stage, several morpho-physiological parameters like shoot length, root length, shoot weight, and root weight are important (Zeng and Shannon. 1998). Different yield and yield contributing characters such as plant height, effective tillers, spikelet sterility (%), yield and yield reduction (%) are pre-requisite to ascertain the effect of salinity at the reproductive stage (Zeng et al. 2001).

Although rice is sensitive to salt, the degree of sensitivity varies depending on the stage of growth and development. It is especially sensitive during the early stages of seedling development and reproduction (Singh et al. 2021). The absence of a link between tolerance at the seedling and reproductive phases provides additional evidence that these two sensitive stages in rice are independent of one another and are governed by distinct gene sets (Singh et al. 2021). Morphological screening at the seedling stage is the pre-requisite for saline tolerant rice variety development because most of the existing cultivar cannot withstand high level of salinity at the vegetative stage.

Fatemadhan, a local landrace selected by a farmer of Bagerhat, Bangladesh from a hybrid rice variety Aftab-5. The most identical morpho-physiological characteristics of this variety are long with spreading flag leaf, larger stem diameter, longer panicle length (36-40 cm), and higher filled grains panicle^{-1}(250-400). Breeding program have to be undertaken to improve several unexpected attributes such as; tall plant, segregation, lower number of tillers, lack of flowering synchronization ,long awn habit, sterility in some growing areas so on. Pure line should be developed before conducting any research using this landrace. It was predicted to have salt and drought tolerant characteristics due to grown in the southern part of Bangladesh. Plant architecture, awnless and growth duration and other attributes can be altered by applying gamma ray based irradiation (Sao et al, 2022). Several potential mutant lines have been developed from Fatemadhan by using gamma irradiation in M_6 generation. Among them 18 lines were screened for salt tolerance in hydroponic condition.

The current study aimed to select the salt tolerant lines in M_6 generation by evaluating morpho-physiological traits at the seedling stage which will be helpful for the development/improvement of rice varieties for salt affected areas in Bangladesh.

2. Materials and Methods

2.1. Plant materials

Local germplasm known as Fatemadhan was collected from the southern part of Bangladesh and exposed to gamma ray irradiation at various doses. Promising mutant lines were selected in the M_6 generation. A total of 20 genotypes were used in this experiment, including 18 M_6 advanced mutant lines and two check varieties (Binadhan-10 and BRRI dhan99).

2.2. Screening for salt tolerance at the seedling stage

The experiment took place in a glass house at BINA, Mymensingh during 2021-22 under hydroponic conditions. Seeds were germinated in petri plates at room temperature and then transferred to Styrofoam sheets floating in trays with Peter's culture solution. Different salinity levels were achieved by dissolving sea salts, and electrical conductivity was monitored with an EC meter. Twenty rice genotypes were grown under various salinity conditions: 0 dSm^{-1} (control), 8 dSm^{-1} (moderate salt stress), 12 dSm^{-1} (high salt stress), and 15 dSm^{-1} (very high salt stress). Salt treatment began at 7 days after sowing and continued until 30 days after sowing. Visual symptom scoring was conducted at 14 and 21 days using the modified IRRI standard evaluation scoring system. After 34 days, ten seedlings from each genotype per treatment were uprooted, and data were collected for morphological parameters (n=20). Measurements included shoot length, root length, root weight, shoot weight, root dry weight, and shoot dry weight.

2.2.1. Evaluation of the genotypes for yield and yield-attributing traits

The second experiment was conducted in the field during 2021-22 to assess yield performance. Data on yield and yield-contributing traits such as effective tillers per plant, filled grains per panicle, unfilled grains per panicle, and yield per plot were recorded.

2.3. Statistical analysis

The first experiment (seedling stage) was conducted under controlled conditions using a completely randomized design (CRD). Data from the second experiment were analyzed using a Randomized Complete Block Design (RCBD) with the MSTAT-C computer package developed by Russell (1986). Treatment means were compared using Duncan's Multiple Range Test. Box plots, radar plots, and heat map clustering were created using R statistics by Friendly (1999). Turkey's multiple comparison test was used to determine significant differences among treatments at the $p<0.05$ level.

3. Results

3.1. Rice genotypes screening based on visual symptoms at seedling stage

Following the visual symptoms of rice seedling at 2 weeks of salt treatment, least SES scored genotypes was selected as tolerant, whereas higher value of SES score considered as susceptible and intermediate values in between susceptible and tolerant genotypes termed as moderately tolerant (Gregorio et al. 1997) (Fig. 9 and 10). Scoring at 14 days after salinization the seedlings of five genotypes such as L-4-P-1-1, LIRG-4Seg-1(1)-3, L-4-P-1(1) and L-4-P-1-P-2 and

Binadhan-10 scored minimum (average 3) and were treated as tolerant; two genotypes *viz*. L-4-P-5(3) and BRRI dhan99 scored maximum (average 6) and were denoted as susceptible; and rest of the genotypes (13)LIRG-2-17Seg-1, LIRG-4 mut 10 (AL)-1, LIRG-4 mut 10 (AL)-2, LIRG-4Seg-1(1)-1, L-4-P-1(1)-2, L-4-P-3(2)-1, L-4-P-5(1), L-4-P-6(3)-1, L-4-P-6(3)-2, L-4-P-6(3), LIRG-2-17Seg-P-1, L-4-P-3(2)-2, L-4-P-4(1) scored medium and were classified as moderately tolerant (Table 1, Figure 9 and 10). Scoring at 21 days after salinization results revealed that 15 genotypes (LIRG-2-17Seg-1, LIRG-4 mut 10 (AL)-1, LIRG-4 mut 10 (AL)-2, LIRG-4Seg-1(1)-1,L-4-P-1(1)-2, L-4-P-3(2)-1, L-4-P-5(1), L-4-P-5(3), L-4-P-6(3)-1, L-4-P-6(3)-2, L-4-P-6(3), LIRG-2-17Seg-P-1, L-4-P-3(2)-2, L-4-P-4(1) and BRRI dhan99 had maximum SES score (average of 7.6) which were denoted as susceptible genotypes; five genotypes such as L-4-P-1-1, LIRG-4Seg-1(1)-3, L-4-P-1(1) and L-4-P-1-P-2 and Binadhan-10 showed minimum (average of 4) SES score and denoted as moderately tolerant (Table 1 and Figure 10).

3.2. Changes in morphological parameters of 20 rice genotypes after salt stress

SL, SW and SDW were remarkably higher at control condition compared to moderate high salt stress and very high salt stress (Fig. 1). At moderate salt stress, minimum change was observed in genotype L-4-P-1(1) (Salt Tolerant Index - 99.32) and maximum change was found in genotype L-4-P-6(3)-2(STI –43.26). In case of shoot length, LIRG-2-17Seg-P-1 genotype exhibited maximum fluctuation (STI-24.98) and minimum in L-4-P-1(1) genotype (STI-98.44). Similar variation was observed in SDW (Data not shown). At very high salt stress, SL reduction ranging from 28.41 cm (LIRG-4Seg-1(1)-1) to 101.67 cm (L-4-P-1(1)). On the other hand, SW fluctuation between genotype to genotype varies from 1.85 (LIRG-2-17Seg-P-1) to 8.19 g. (L-4-P-1(1)). Besides, maximum decline in SDW was found in LIRG-2-17Seg-P-1 (4.27 g) and the lowest in L-4-P-1-P-2 mutant (1.49 g) respectively (Data not shown). According to the morpho-physiological parameters of shoot, STI values were higher in L-4-P-1(1) and L-4-P-1-P-2 mutant indices greater salinity tolerance. Like shoot parameters different root traits e.g. RL, RW and RDW were relatively higher at control condition weighed to moderate, high salt stress and very high salt stress. At high salt stress, higher Root length (RL) was observed in L-4-P-1(1) genotype (84.49 g.). The two parameters Root weight (RW) and Root Dry weight (RDW) also pursued the similar leaning and were notably diminished under very high salt stress when weighed to the control. The Lowest RW and RDW were found in LIRG-2-17Seg-1 (0.24 g) and LIRG-4Seg-1(1)-1(0.1 g) respectively. Root dry weight (RDW) declined radically, and deviates from 5-7% compared to RDW at ideal condition. At very high salt stress, the lowest RL, RW and RDW were obtained in LIRG-4 mut 10 (AL)-1 genotype counting as 8.32 cm (STI-35.67), 0.35 g (STI-19.33), 0.54 (24.14 g) respectively in contrast highest RL, RW and RDW recorded in L-4-P-1(1) mutant found as 17.81 cm (STI-76.82), 1.87 g. (STI-73.76) and 0.47 (STI-66.88) respectively (Data not shown here).

Table 1. Scoring of 20 genotypes based on SES system

Time of salinity impose	Line/parent	SES score	Level
14 days	L-4-P-1-1, LIRG-4Seg-1(1)-3, L-4-P-1(1), L-4-P-1-P-2 and Binadhan-10 (check), L-4-P-6(3)-2, L-4-P-6(3), L-4-P-4(1)	3	Tolerant
	LIRG-4 mut 10 (AL)-1, LIRG-4 mut 10 (AL)-2, LIRG-4Seg-1(1)-1, L-4-P-1(1)-2, L-4-P-3(2)-1, L-4-P-5(1), L-4-P-6(3)-1, LIRG-2-17Seg-P-1, L-4-P-3(2)-2, LIRG-2-17Seg-1, BRRI dhan99 and L-4-P-5(3)	5	Moderately Tolerant
21 days	L-4-P-1(1), L-4-P-1-P-2 and Binadhan-10 (check), L-4-P-1-1 and LIRG-4Seg-1(1)-3	3	Tolerant
	LIRG-4 mut 10 (AL)-2, LIRG-4Seg-1(1)-1, LIRG-2-17Seg-P-1, L-4-P-3(2)-2, L-4-P-4(1) and L-4-P-6(3)-1, LIRG-2-17Seg-1, LIRG-4 mut 10 (AL)-1, L-4-P-1(1)-2, L-4-P-6(3)-2, L-4-P-6(3), BRRI dhan99(Check), L-4-P-3(2)-1, L-4-P-5(1), L-4-P-5(3) and L-4-P-5(3)	7	Susceptible

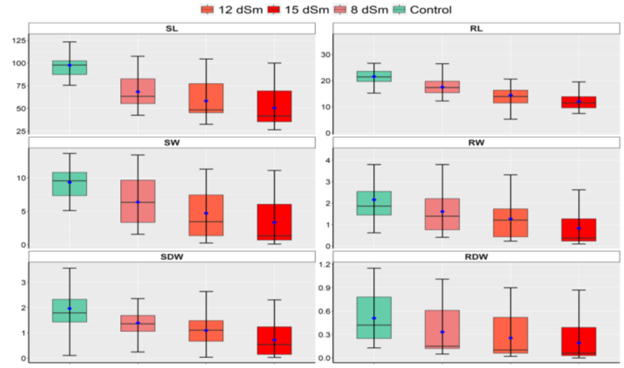

Figure 1. Box and whisker plots for growth related parameters at seedling stage exhibiting inter specific difference and the effect of different (8 dS/m, 12 dS/m and 15 dS/m) salinity treatments on the mean of SL- Shoot length, RL-Root length, SW-Shoot weight, RW-Root weight, and SDW- Shoot dry weight and RDW- Root dry weight

3.3. Screening salt tolerant rice genotypes through heatmap analysis

The STI (stress tolerance index) values obtained from studied traits of the rice genotypes were normalized and clustered. Three-row clusters (cluster numbers were determined by the machine language of gap statistic) were obtained at the genotype level (row cluster-1, 2, 3) and two-column clusters (column cluster-1 and 2) were found at the trait level. The tolerance level of genotypes after imposed salinity is evident at row clusters at different salinity level 8 dS/m (Figure 2), 12 dS/m (Figure 3) and 15dS/m (Figure 4). The genotypes lie in the green colour shades, considered as having more salinity tolerance level and increases susceptibility after reaching to the yellow and red colored areas. In that perspective, the mutants L-4-P-1-1, LIRG-4Seg-1(1)-3, L-4-P-1(1) and L-4-P-1-P-2 exhibited higher tolerance whereas the lines L-4-P-5(3), L-4-P-3(2)-2, LIRG-4 mut10 (AL)-1 and L-4-P-4(1) were moderately tolerant and rest of those had higher susceptibility at all imposed saline doses (Figure 2, Figure 3 and Figure 4).

To confirm the robust hierarchical result, radar plot clustering was performed for all three level of salinity (8, 12 and 15 ds/m) treatment. Tolerant, moderately tolerant and susceptible genotypes were grouped into cluster-1 (green colored), cluster-2 (red colored) and cluster-3 (blue colored) respectively at radar plot chart (Figure 5, Figure 6 and Figure 7). The mutants L-4-P-1-1, LIRG-4Seg-1(1)-3, L-4-P-1(1) and L-4-P-1-P-2 were categorized into the cluster-1, the mutants L-4-P-5(3), L-4-P-3(2)-2, LIRG-4 mut10(AL)-1 and L-4-P-4(1) is in cluster-2 and rest of the variety grouped into cluster-3. Cluster-1 covered more area of the plot and having maximum value of STI while cluster-2 encircled less area than the cluster-1. The lowest covering of STI value shows higher susceptibility against salinity.

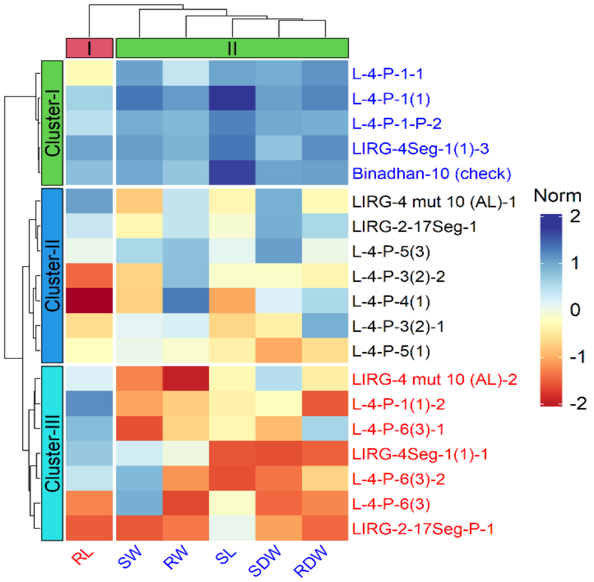

Figure 2. Robust hierarchical clustering indicates trait and genotypes association expressed as STI values of the traits at 8 dS/m saline condition

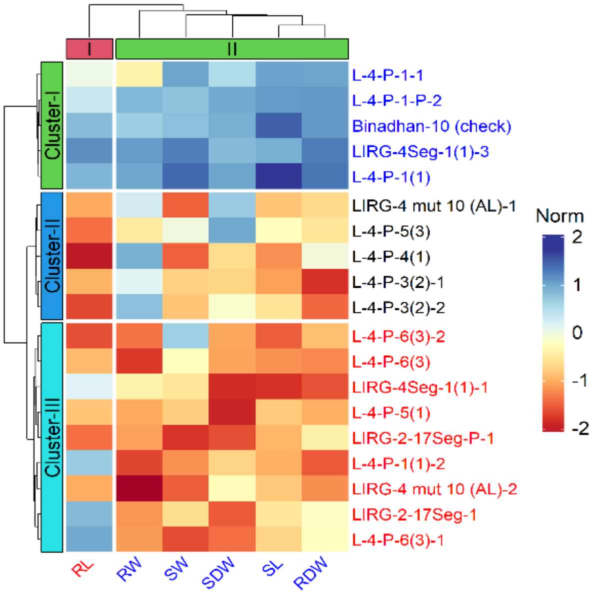

Figure 3. Robust hierarchical clustering indicates trait and genotypes association expressed as STI values of the traits at 12 dS/m saline condition

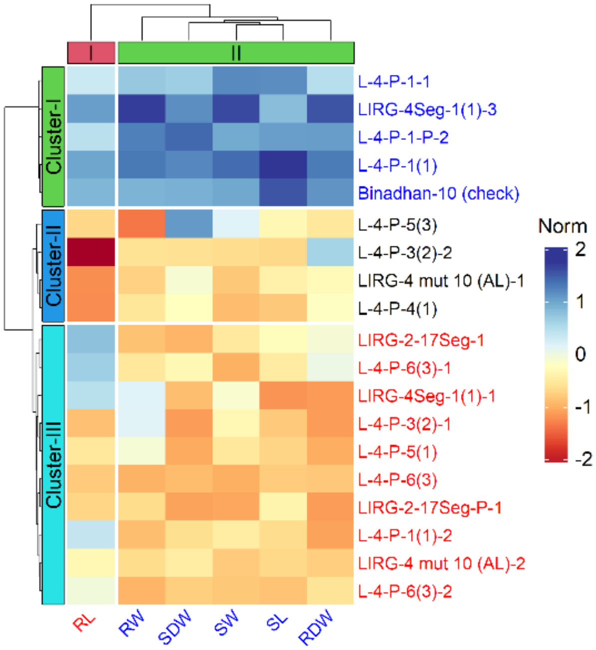

Figure 4. Robust hierarchical clustering indicates trait and genotypes association expressed as STI values of the traits at 12 dS/m saline condition

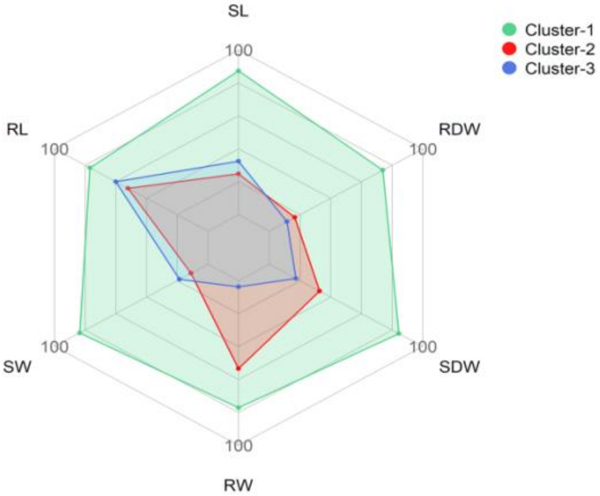

Figure 5. Clustering through radar plot at 8 dS/m saline condition

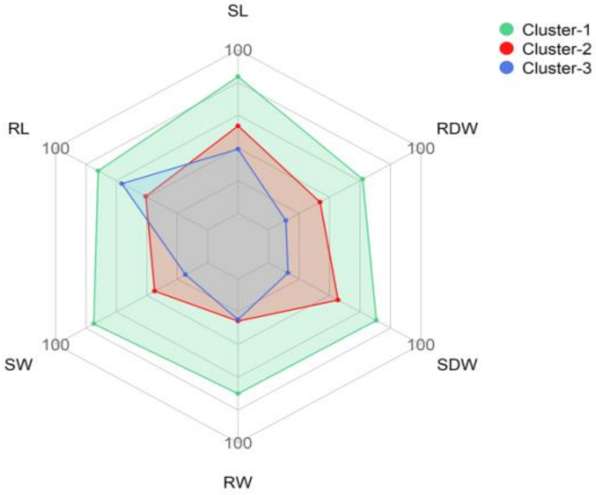

Figure 6. Clustering through radar plot at 12 dS/m saline condition

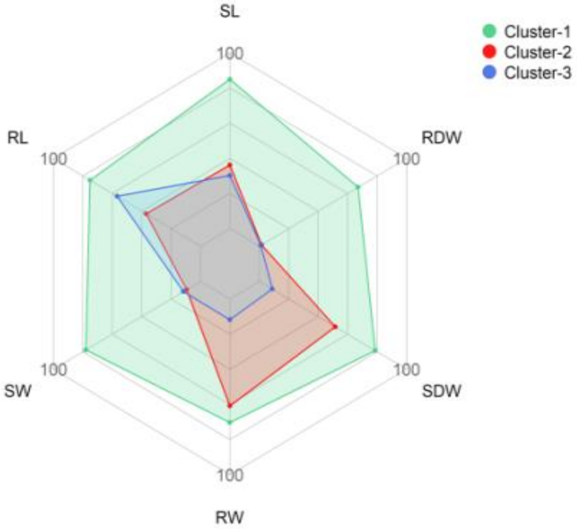

Figure 7. Clustering through radar plot at 15 dS/m saline condition

3.4. PCA based screening of salt-resistant rice genotypes at seedling stage

A PCA biplot analysis can be used to choose traits that can be differentiated into main groups and subgroups based on homogeneity and dissimilarity. In our data set, two sets of traits were found in the PCA biplot considering both PC1 and PC2 simultaneously (Figure 8). The SDW, SW, RW and RDW were clustered in group I, while RL were in group II which also be proved by robust hierarchical clustering (Figure 2, 3 and 4). Interestingly, the PCA biplot revealed that group I traits, the major contributors in PC1, explains 73.2% variation, moderately tolerant and susceptible genotypes lie in this area (Figure 8). The traits of group II contributed to PC2 explain also 9.4% variation and tolerant genotypes were found in this orbit. PCA-biplot also indicated the cluster centroids (the multi-dimensional average of the cluster) and the approximation of distances among them (Figure 8)

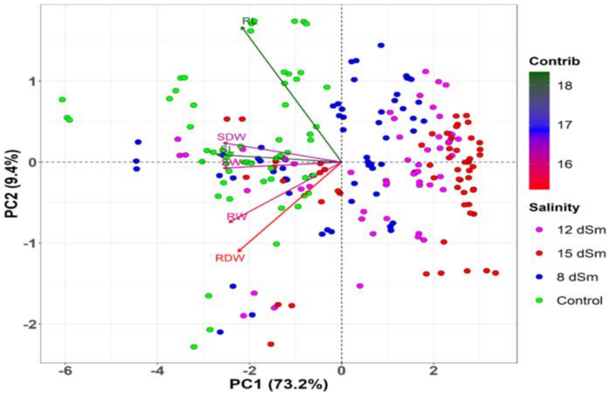

Figure 8. PCA-Biplot of seedling traits coupled with rice genotypes. Genotypes dispersed in different ordinates based on the dissimilarity among them. The length and color intensity of a vector in the biplot indicate the quality of representation and the contribution of the traits, respectively, on the principal components. The angles between the vectors derived from the middle point of biplots exhibit positive or negative interactions of studied traits. Bigger circles indicate the centroid of the corresponding cluster. (SL—shoot length; RL—root length; SW—shoot fresh weight; RW—root fresh weight; SDW—shoot dry weight; RDW—root dry weight)

3.5. Characterization of the genotype for yield and yield attributing traits

To assess the agronomic performance with yield characters of the mutant lines, an experiment was conducted in natural growing condition at BINA Substation, Magura.

The line LIRG-4Seg-1(1)-1 exhibited highest plant height (128.6 cm) whereas and LIRG-2-17Seg-P-1 line showed lowest PH (Table 2). Significant variations were found in effective tillers among the mutants. Highest tiller plant^{-1} was found in Binadhan-10 (18.7) while in L-4-P-5(1) mutant lowest effective tillers plant^{-1} (3.4) was found. Longest panicle length (28.9 cm) was found in LIRG-4Seg-1(1)-3 mutant while lowest panicle length was observed in check variety Biandhan-10 (23.5 cm). Most of the mutants performed better in relation to yield. Among seedling stage salt tolerant mutants, LIRG-4Seg-1(1)-3 showed highest yield (7.67 t/ha) with longest panicle length (28.9 cm). In normal condition, the yields of L-4-P-1-1, LIRG-4Seg-1(1)-3, L-4-P-1(1) and L-4-P-1-P-2 lines are 5.8, 7.6, 3.3, 4.0 t/ha respectively.

4. Discussion

The stress tolerance index (STI) can be used to identify morpho-physiological changes in genotypes under both stressed and non-stressed conditions (Hedrich, 2018). Decreased root growth and increased shoot growth may improve salt tolerance by limiting the flow of toxic ions towards the shoot, thus delaying the onset of threshold tolerance (Maggio et al., 2007). Our study showed a significant decrease in shoot dry weight and root dry weight under different salt stress conditions. Puvanitha and Mahendran (2017) also found similar results. Shoot length, shoot dry weight, and root length decrease with increasing salt stress (Jamil et al., 2007). The decline in shoot and root development may be due to the lethal effects of NaCl and unequal nutrient uptake by seedlings (Datta et al., 2009). The decrease in shoot dry weight could also be linked to a reduced rate of leaf production, resulting in fewer leaves, decreased photosynthesis, and dry matter development (Neumann, 1997). The decline in root dry weight may be a result of specific ion and osmotic effects of Na+ and Cl- (Taffouo et al., 2010). Decreased water potential in the soil solution may lead to water shortage, reducing root water uptake. Ion toxicity may have affected the plant's water uptake, resulting in root injury and death (Netondo et al., 2004).

Dendrogram analysis is a grouping method based on similarity or dissimilarity among genotypes in a population (Widyastuti et al., 2016). Cluster heatmap or clustergram can address the limitations of dendrogram clusters by combining two dendrograms in the same dimension attached to heatmap analysis (Schonlau, 2002). This combination reveals a relationship pattern among genotypes and characters in color intensity, making it easy to understand (Bowers, 2010). Higher color intensity indicates that a genotype has a stronger response than others in a particular character (Lee et al., 2016). This analysis can be used to identify traits with good tolerance under salinity screening.

Principal Component Analysis (PCA) is a powerful statistical procedure for reducing the dimensions of variables and extracting meaningful information from a highly correlated dataset (Bahrami et al., 2014). While the angles between vectors of traits in biplot analysis may not precisely correlate with correlation coefficients (Abdi and Williams, 2010), our results showed that correlations of trait pairs were well-coordinated with vector angles in the PCA biplot (Figure 8). PCA includes all parameters for analysis and distinguishes genotypes under stress conditions (Jianjie et al., 2013), allowing for the identification of potential groups and associations between accessions and traits (Martinez-Calvo et al., 2008). A three-way PCA biplot effectively distinguishes germplasm as salt-tolerant or salt-sensitive genotypes (Raza et al., 2017).

Screening at the seedling stage may sometimes yield inaccurate results in selecting salt-resistant genotypes. Crops tend to gain resistance during vegetative growth, become sensitive during the reproductive stage, and regain resistance in later stages of maturity (Rao et al., 2008; Lafitte et al., 2006). Significant variability among yield-attributing traits was also reported in the studied mutants. Similar to our results, variation in tiller number in different rice mutants has been reported by others (Abdullah et al., 2001). Differences in panicle length, filled grains per panicle, and unfilled grains per panicle indicate variation caused by mutation and the development of different stable mutant lines (Nicolas et al., 1994).

Sl. No	Accession number	Plant height (cm)	Effective tillers	Panicle length (cm)	Filled grain panicle^{-1}	Unfilled grain panicle^{-1}	Grain yield (t/ha)
1	LIRG-2-17Seg-1	109.6 f-h	5.0 d-h	28.6 ab	236 e	34.3 c-e	5.7 fg
2	LIRG-4 mut 10 (AL)-1	112.6 d-g	8.0 bc	24.6 f-h	191.3 i	29.3 c-e	6.5 e
3	LIRG-4 mut 10 (AL)-2	115.0 de	8.1 bc	25.0 e-h	256.6 cd	25.2 e	6.6 e
4	L-4-P-1-1	123.0 b	5.8 c-h	23.6 gh	199.5 hi	30.6 c-e	5.8 f
5	LIRG-4Seg-1(1)-1	128.6 a	7.8 bc	23.8 gh	193 hi	29.6 c-e	7.4 b
6	L-4-P-1(1)-2	108.0 g-i	4.5 e-h	27.6 a-c	285.0 b	57.2 b	6.7 e
7	LIRG-4Seg-1(1)-3	111.5	9.0 b	28.9 a	210.0gh	39.3 cd	7.6 b
8	L-4-P-3(2)-1	121.8 bc	8.0 bc	25.0 f-h	206.6 hi	32.6 c-e	7.4b
9	L-4-P-5(1)	103.8	4.4 f-h	27.6 a-c	229.0 ef	77.3 a	3.93
10	L-4-P-5(3)	114.0 def	6.3 c-f	25.9 c-f	210.6gh	27.40 de	6.3 e
11	L-4-P-6(3)-1	114.2 def	7.4 b-d	26.9 b-e	225.3 e-g	39.0 cd	9.5 a
12	L-4-P-6(3)-2	110.6 e-h	6.4 c-f	25.9 c-f	210.6 f-h	35.3 c-e	6.8 c-e
13	L-4-P-6(3)	112.6 d-g	6.4 c-f	26.4 c-f	195.0 hi	23.8 e	7.4 b
14	L-4-P-1(1)	110.3 e-h	4.6 e-h	25.4 d-g	262.0 c	62.3 b	3.3 i
15	L-4-P-1-P-2	117.4 cd	3.8 gh	26.4 c-f	264.3 c	37.0 c-e	4.0 h
16	LIRG-2-17Seg-P-1	97.66 j	7.0 b-e	27.2 a-d	194.3 hi	32.7 c-e	7.3 bc
17	L-4-P-3(2)-2	107.3 hi	6.0 c-g	28.4 ab	312.3 a	62.0 b	7.2 b-d
18	L-4-P-4(1)	112.3 e-g	3.4 h	28.4 ab	240.3 de	42.0 c	5.2 g
19	Binadhan-10 (check)	91.64 k	18.7 a	23.5 h	52.26 j	23.9 e	6.8 de
	CV (%)	2.62	21.69	4.21	5.10	21.28	4.68

Here, PH-Plant height, ET-Effective tiller, PL-Panicle length, FGP-Filled grains/panicle, UGP- Unfilled grains/panicle and GY- Grain yield. Different letters are significant at 5% level of probability following Turkey's method.

Plate (a) Plate (b) Plate (c) Plate (d)

Figure 9. (a) indicates shoot and root retardation after 14 days of salt impose ; (b) Root length of tolerant mutant; (c) Growth retardation under 15dS/m salt stress between tolerant and susceptible mutants respectively after 14 days; (d) Growth retardation under 12dS/m salt stress between tolerant and susceptible mutants respectively after 14 days

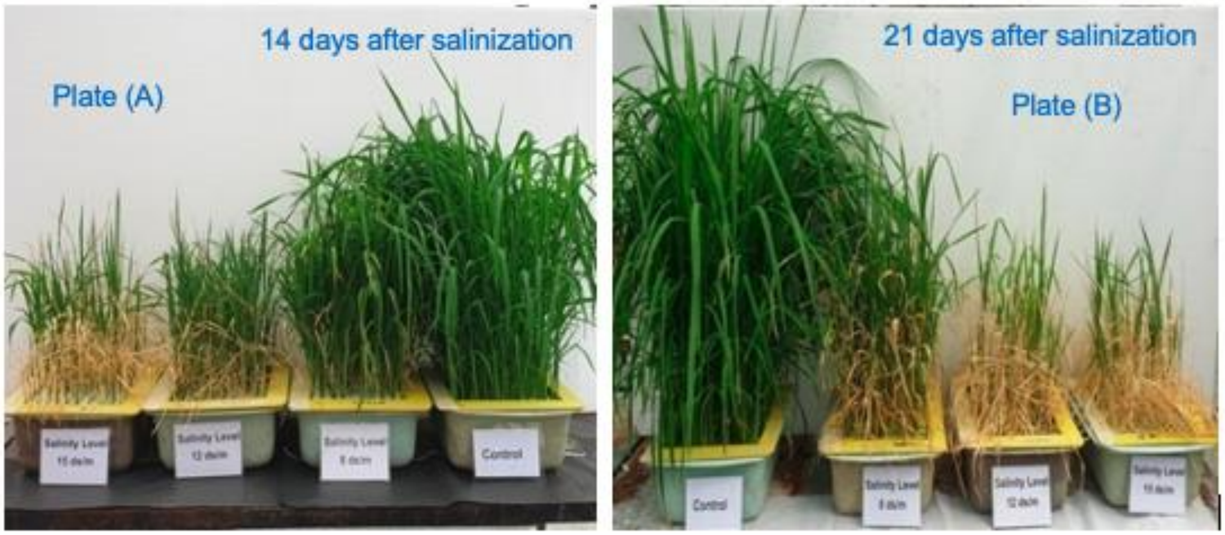

Figure 10. Phenological appearance of rice seedlings grown under 8 dS/m, 12 dS/m and 15 dS/m salinity stress in hydroponic peter solution for 14 days (Plate A) and 21 days (Plate B), respectively

5. Conclusion

The imposed salinity stress at the seedling stage brings about a significant decrease of shoot and root related parameters. By considering the SES score, heatmap and Radar plot graph, four genotypes L-4-P-1-1, LIRG-4Seg-1(1)-3, L-4-P-1(1) and L-4-P-1-P-2 found as salt tolerant at the seedling stage. The PCA biplot confirms limited increase or decrease of the contributing salt tolerance traits in term of above stated genotypes. Among the four potential lines screened at seedling stage, two lines L-4-P-1-1, LIRG-4Seg-1(1)-3 gave higher yield (5.8 and 7.6 t/ha) in normal condition. However all the selected mutants at the seedling stage should be screened for salinity tolerance at the reproductive stage to develop a saline tolerant variety. Moreover, extensive research is needed with these lines for specific gene (s) isolation or QTL conferring salt tolerance in vegetative and reproductive growth phases and also using these lines as donar parents in salt tolerant rice breeding program.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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