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Phenotypic Screening of Rice Varieties for Tolerant to Salt Stress at Seed Germination, Seedling and Maturity Stages

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ABSTRACT: *Salinity affects all growth stages of rice in varying degrees starting from germination up to maturation. The present study was conducted to screen the commonly cultivated rice varieties in salinity affected areas in Sri Lanka against salt stress. Screening of 21 improved rice varieties and check variety Pokkali was performed at seed germination, seedling and maturity stages in petri dishes, in a hydroponic system and in soil filled pots, respectively. Rice varieties were tested for ability to sustain seed viability, growth performance of seedling and plant survival, growth performance and grain yield reduction at maturity under salt stress. At germination stage, Bg 406 and Pokkali were identified as highly tolerant rice varieties whereas, At 402 showed the highest tolerance level followed by Pokkali, Bg 369 and At 354 at seedling stage. Based on the mean separation on survival of plants per pot at 12 dS/m and 8 dS/m, three groups (highly tolerant, tolerant and susceptible) could be identified. Percentage reduction increased with increasing salt levels for most of the parameters tested (PH, PL, RDW, SDW, PWT, PNO and YLD). Based on the phenotypic observations Pokkali was identified as high salinity tolerant variety whereas Bg 369, At 354, At 402 were identified as salinity tolerant varieties at maturity stage.*

Keywords: *Phenotypic response, rice, salt stress, varieties*

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important cereal crops and serves as the staple food for nearly half of the world's population (Mohammadi *et al*., 2010). However, the productivity of rice is greatly affected due to soil salinity which is the second most widespread soil problem next to drought in rice growing areas of the world (Sabouri, 2008; Islam *et al*., 2011). There are two types of salinity; inland salinity which is caused by irrigation practices with salty water and coastal salinity which is mainly caused by high tides of ocean in the coastal region (Ganie *et al*., 2016). Approximately 21.5 million hectares of arable land in Asia are facing salinity problem and the estimated crop loss is up to 50% of fertile land by the 21st midcentury (Nazar *et al*., 2011; Huyen *et al*., 2013). Despite these challenges rice production has to be increased by at least 25% by 2030 to keep pace with predicted population growth (Li *et al*., 2014).

Rice is very sensitive to salinity during early seedling and reproductive stages. However, it is comparatively tolerant to salinity during germination, active tillering and at maturity (Lafitte

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et al., 2004). Salinity affects yield components such as panicle length, spikelet number per panicle, grain yield and also delays panicle emergence and flowering (Flowers and Yeo, 1981; Flowers and Yeo, 2000). There are some traditional cultivars and landraces which are naturally tolerant to salt stress due to their adaptation to thrive on salt affected land for generations. However, they generally have poor agronomic characteristics such as tall plant stature, poor grain quality, low yield, and photosensitivity (Gregorio *et al*., 1997; Thiruchelvam and Pathmarajah, 1999). One of the traditional cultivars, *Pokkali*, has been recognized for having higher degree of salt tolerance than tolerant cultivars and thus used as a high potential salt tolerant donor for breeding (Bonila *et al*., 2002).

In agriculture, a soil having a salt concentration exceeding an electrical conductivity (EC) value of 4 dS/m is classified as saline (USDA-ARS, 2008). It has been observed that the yield of most crops is reduced at salinity levels above 4 dS/m, which is equivalent to 40 mM NaCl and an osmotic pressure of 0.2 MPa. In Sri Lanka, approximately 13% of the irrigated lands are affected by salinity stress (Gregorio *et al*., 1997) and this percentage increases gradually in both coastal regions and inlands (Thiruchelvam and Pathmarajah, 1999). Salinity is gradually increasing in the rice lands of Sri Lanka both in the coastal regions and inlands. Inland salinity can be observed in Anuradhapura , Polonnaruwa, Puttalam, Hambanthota and Ampara districts and costal salinity is present in Mannar, Puttalam, Jaffna, Trincomalee, Ampara, Hambantota, Galle, Kalutara and Matara districts (Sirisena and Herath, 2009).

Most of the rice varieties are extremely sensitive to salinity during young seedling stage and early development stage (Heenan *et al*., 1988). Agronomical practices such as improved field drainage, maintenance of adequate amount of water around 2 to 3 cm height until early reproductive stage (Costa *et al*., 2012), establishment of crop by transplanting (Thiruchelvam and Pathmarajah, 1999) and use of organic manure instead of inorganic fertilizer are used to address salinity. However, these practices are not cost effective, efficient and favorable for a long-term solution. Hence, cultivation of durable resistant rice varieties appears more appropriate as a long-lasting solution. Therefore, identification of salt tolerant varieties and introgression of their salt tolerant characteristic into high yielding cultivars is necessary in order to utilize salt affected lands for sustainable rice cultivation. Hence, the present study was conducted to screen twenty two popular rice varieties grown in salinity affected areas in Sri Lanka against salt stress and to identify mega varieties that can address the problem of salt stress.

MATERIALS AND METHODS

Plant Materials

This study was carried out at the Rice Research and Development Institute, Batalagoda (RRDI, Bg) which comes under the low country intermediate zone. Seeds of twenty-one improved rice varieties namely Bg 379-2, Bg 450, At 402, Bg 403, Bg 406, Bg 94-1, Bg 352, At 353, At 354, Bg 357, Bg 358, Bg 359, Bg 360, At 362, Ld 365, Bg 366, Bg 369, Bg 300, Bg 4-91, At 307, At 308 and Pokkali as salinity resistant check variety were collected from rice research station Bathalagoda. The varieties were selected based on the coverage of land area affected by salinity.

Response to salt stress at seed germination stage

Varieties were tested for their ability to sustain seed viability under high salt concentrations to screen tolerance to salinity as proposed by Abesiriwardena (2004). Sodium chloride solutions were prepared and fifty seeds from each cultivar were soaked in the solutions with electrical conductivity (EC) levels of 0, 40, 45 and 50 (dS/m) for 9 and 12 days in petri dishes. Seeds were taken out after completing the soaking period and washed thoroughly with distilled water to remove the salt deposited around seeds before placing them on wetted blotting paper in petri dishes for germination. Seeds were allowed to germinate under ambient temperature. The average temperature inside the laboratory during the study period was 27 $^{\circ}$C. Number of germinated seeds was counted for each variety in each replicate after 5 days. The experiment was a 22 (variety) × 4 (salt concentrations) × 2 (soaking periods) three factor factorial laid out in a Completely Randomized Design (CRD) with 2 replications. Experiment was repeated twice to determine the consistency of results.

Response to salt stress at seedling stage

The experiment was conducted in the green house of the RRDI in *Yala* 2014. At the seedling stage, varieties were screened for salt tolerance in a hydroponic system using International Rice Research Institute's (IRRI) standard protocol (Gregorio *et al*., 1997). Three replications were used in the experiment. The evaluation was performed using the nutrient solution proposed by Yoshida *et al.,* (1976). Sterilized seeds were placed in Petri dishes with moistened filter papers and incubated for 48 hours to germinate (Gregorio et al., 1997). Two pre-germinated seeds were planted per hole on the Styrofoam seedling float. Each replicate consisted of 8 such holes. The radicle was inserted through the nylon mesh. During this process, damage caused to radicle will interferes with the inherent of salt tolerance mechanism of rice. Therefore, seedlings were allowed 3 days to repair any root damage. After the recovery seedlings were allowed to grow under normal nutrient solution up to 5 days. After well establishment of seedlings, nutrient solution was salinized by adding NaCl while stirring up to the 6 dS/m and after 3 days increased it in to 12 dS/m. Distilled water was used in making up Yoshida solution as local tap water may result in precipitation of minerals and will alter mineral concentrations that may affect salt sensitivity. Due to evaporation and transpiration there was a loss of solution volume changing the pH. Every two days the volume was brought back to the level of touching the netting in the Styrofoam seedling float and the pH adjusted to 5 by adding 1% HCl or 5 % NaCl. Solution was changed by lifting off the Styrofoam seedling float and placing them temporarily onto an empty basin and pouring the hydroponics solutions back into a big container where the bulked solution is pH adjusted for the whole experiment in one step. Once adjusted, the solution was re-distributed into the test containers and the seedling platform returned. These operations also helped to aerate the hydroponics solution. The modified standard evaluation system (SES) was used in rating the visual symptoms of salt toxicity (Gregorio et al., 1997). Visual scoring of salinity tolerance was performed as shown in Table 1. Scoring discriminated the susceptible from the tolerant and the moderately tolerant genotypes. Initial and final scorings were performed 10 days and 16 days after salinization, respectively.

Response to salt stress after seedling stage

An experiment was conducted in *Maha* 2014/15 in the green house at RRDI. The varieties were evaluated for their tolerance to salinity in a pot experiment based on the performance of the mature plants under saline conditions. Soil was collected; air dried, sieved through 4 mm sieves and filled into pots at the rate of 5 kg per pot. NaCl was added at the rates of 0, 6, 12 and 18 g per pot to obtain the salinity levels of 0, 4, 8, 12 dS/m, respectively. Soil in pots was mixed with water and allowed to settle for 2 weeks. Fertilizers were added and mixed with the soil at the time of planting at the rate of 0.3 g of urea, 0.75 g of concentrated super phosphate and 25 g of muriate of potash. Three weeks old rice seedlings of 22 varieties were planted at the rate of 3 plants per pot. Saturated moisture level was maintained in each pot throughout the growing season until maturity. The experiment was a 22 (variety) × 4 (salinity levels) two factorial laid out in a CRD with three replications. Data were recorded for survival of plants per pot, plant height (PH), panicle length (PL), root dry weight (RDW), shoot dry weight (SDW), panicle weight (PWT), panicle number per plant (PNO), and grain yield per plant (YLD).

Table 1. Modified Standard Evaluation System (SES) of visual salt injury scoring at seedling stage

Score	Observation	Degree of Tolerance
100%	Normal growth, no leaf symptoms	Highly tolerant
75%	Nearly normal growth, but leaf tips or few leaves whitish and rolled	Tolerant
50%	Growth severely retarded; most leaves rolled; only a few are elongating	Moderately tolerant
25%	Complete cessation of growth; most leaves dry; some plants dying	Susceptible
0%	Almost all plants are dead or drying	Highly susceptible

Source: Gregorio *et al.,* (1997)

Statistical analysis

Germination percentages of the selected rice varieties at seed germination stage were analyzed by using SAS 9.1.3 (SAS Institute Inc, USA). To check the difference among rice varieties for salinity tolerance at seed germination stage and seedling stage, analysis of variance was performed using Proc GLM procedure followed by the Duncan Multiple Range Test (DMRT). Plant survival percentage was calculated by using survival plants per pot. For the preparation of dendograms, PH, PL, RDW, SDW, PWT, PNO, and YLD were considered. The cluster analysis was performed using a complete linkage method (Korenius *et al*., 2007).

RESULTS AND DISCUSSSION

Response to salt stress at seed germination stage

Two way interaction effects of variety × soaking period, variety × salt concentration and soaking period × salt concentration were found to be significant at 0.05 probability levels. The significant interactions using response curves were observed and 12 days of soaking and 50 dS/m salt concentration were identified as the best soaking period and salt concentration combination to clearly separate varieties for different groups based on reaction to salt stress. According to the mean separation, selected rice varieties can be categorized in to 6 groups with their response to salinity (Table 2).

Table 2. Rice varieties grouped under different clusters at seed germination stage

Cluster	Salinity tolerance	Cluster varieties	Germination rate
VI	Highly tolerant	Bg 406[a]	>70%
V	Tolerant	*Pokkali*[b]	*51-70%*
IV	Moderately tolerant	Bg 450[c]	31-50%
III	Moderately Susceptible	At 354[d] ,Bg 358[d]	21-30%
II	Susceptible	Ld 365[de], Bg 300[de], Bg 4-91[de], Bg 379-2[de], At 402[def], Bg 403[def], Bg 366[def]	11-20%
I	Highly susceptible	Bg 352[fg], At 308[fg], Bg 94-1[g], At 362[g], Bg 359[g], Bg 357[g], Bg 369[g], Bg 360[g], At 353[g], At 307[g]	0-10%

Note: the mean followed by the same letter are not significantly different at p=0.05

Response to salt stress at seedling stage

Based on the mean separation of score percentage, 16 days after salinization, varieties showed significant differences. The varieties were categorized in to five level of stress response (Table 3). At 402 was identified as the highly tolerant variety among the tested varieties whereas *Pokkali,* At 354 and Bg 369 were identified as salt tolerant varieties at the seedling stage.

Table 3. Grouping of different rice varieties based on their reaction to salt stress at the seedling stage

Varieties	Degree of tolerance
At 402[a]	Highly tolerant
Pokkali[b], At 354[b] , Bg 369[b]	Tolerant
At 353[c], Bg 379-2[c], Bg 403[c], Bg 406[c]	Moderately tolerant
At 307[d]	Susceptible
Bg 358[e], Bg 4-91[e], Bg 360[e], Bg 362[e], Bg 365[e], Bg 366[e], Bg 352[e], Bg 357[e], Bg 308[e], Bg 359[e], Bg 450[e], Bg 94-1[e], Bg 300[e]	Highly susceptible

Note: the mean followed by the same letter are not significantly different at p=0.05

Reaction to salt stress after seedling stage

The two way interaction effect of variety × salt concentration were significant with PH, PL, RDW, SDW, PWT, PNO, and YLD. Influence of different salinity levels on the height of the rice varieties was significant at the 8 dS/m and 12 dS/m. The influence of salinity on the height has been reported by many researchers (Gridhar, 1988). The results showed that the height of rice at different levels of salinity (3.6 to 8.3 dSm^{-}1) decreased but not statistically significant (Gridhar, 1988). PL of the tested varieties decreased with increasing salt levels. Influence of different salinity levels on the PL of the rice varieties was significant at the 8 dS/m and 12 dS/m. Highly tolerant varieties have lower reduction percentage (Table 4) of PL compared to susceptible varieties.

Table 4. Range of the percentage reduction of the phenotypic traits at 8 dS/M and 12 dS/m

Level of tolerance	EC level	Range of the percentage reduction						
		PH (cm)	PL (cm)	RWD (g)	SWD (g)	PWT (g)	PNO	YLD (g)
Highly tolerant	8 dS/m	0-10	0-2	40-60	0-50	80-95	0-25	70-88
	12 dS/m	0-20	2-10	50-75	0-70	90-95	0-25	85-90
Tolerant	8 dS/m	10-15	2-15	60-70	50-70	80-95	25-50	70-90
	12 dS/m	20-30	10- 20	75-80	70-90	80-95	25-65	85-95
Moderately tolerant	8 dS/m	10-15	15-20	70-99	70-80	80-95	65-70	75-90
	12 dS/m	100	100	100	100	100	100	100
Moderately susceptible	8 dS/m	15-20	20-25	75	75-85	80-95	50-80	80-90
	12 dS/m	100	100	100	100	100	100	100
Susceptible	8 dS/m	100	100	100	100	100	100	100
	12 dS/m	100	100	100	100	100	100	100
Highly susceptible	8 dS/m	100	100	100	100	100	100	100
	12 dS/m	100	100	100	100	100	100	100

RDW of the tested varieties decreased with increasing salt levels. Influence of different salinity levels on the RDW of the rice varieties was significant at the 8 dS/m and 12 dS/m. Salt sensitive varieties showed higher percentage of reduction in RDW compared to tolerant varieties. SDW of the tested varieties decreased with increasing salt levels. Influence of different salinity levels on the SWD of the rice varieties was highly significant at the 8 dS/m and 12 dS/m. The influence of salinity on the dry weight of straw was reported in many studies (Kavosi, 1995; and Zeng and Shannon, 2003), whereas some research showed that salinity (3.6 to 8.3 dSm-1) does not affect the weight of straw before heading stage (Zeng and Shannon, 2003).

PW of the tested varieties decreased with the increasing salt levels. Influence of different salinity levels on the PW of the rice varieties was significant at the 8 dS/m and 12 dS/m. Salinity has a very significant influence on grain yield (Yeo and Flowers, 1986). PNO of the tested varieties decreased with the increasing salt levels. Influence of different salinity levels on the PNO of the rice varieties was significant at the 8 dS/m and 12 dS/m. High influences of salinity on the number of filled panicles were reported by several studies (Aschi *et al.*, 2001; Beatriz *et al.,* 2001). Salt sensitive varieties showed higher percentage of reduction in PNO compared to tolerant varieties. YLD of the tested varieties decreased with increasing salt levels. Influence of different salinity levels on the YLD of the rice varieties was significant at the 8 dS/m and 12 dS/m. High influence of salinity on rice yield and rice sensitivity to salinity was reported by several researchers (Kadda *et al.*, 1973; Muns and Termaat, 1986; Shahdy, 1994). Percentage reduction of plant PH, PL, RDW, SDW, PWT, PNO, and YLD 12 dS/m salinity level were present in Figure 1. Out of all tested varieties *Pokkali*, Bg 369, At 354 and At 402 can be clearly separated out as salinity tolerant varieties.

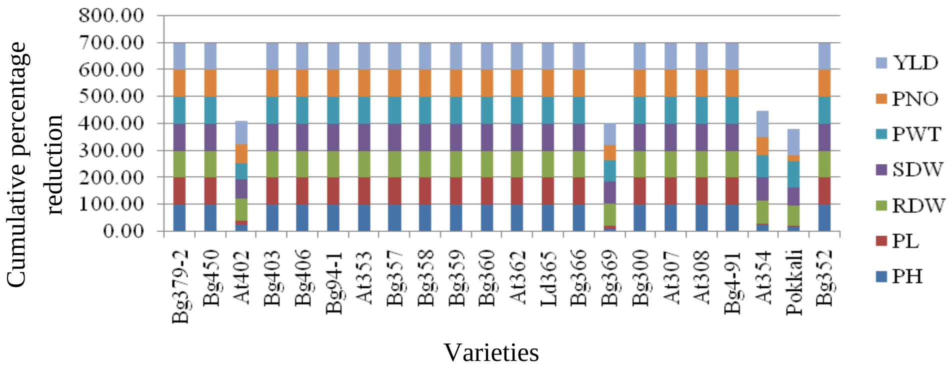

Figure 1. Cumulative percentage reduction in PH, PL, RDW, SDW, PWT, PNO and YLD at 12 dS/m salinity level

Clustering of varieties based on plant survival

According to the data analysis of survival percentage of plants per pot, 8 dS/m and 12 dS/m salinity levels were significantly different from the other levels of salinity. Highest significant difference obtained from the 12dS/m salinity level at 0.05% probability level. According to the mean separation at the 12dS/m salinity level At 402, Bg 369, *Pokkali* and At 354 showed significantly higher salt tolerance among selected rice varieties. Therefore they can be identified as salt tolerant varieties. At the 8 dS/m salinity level, the varieties Bg 357, At 362, At 308, At 307, Bg 300, Bg 360, Ld 365 and Bg 352 were significantly different from the rest of varieties for the lowest rate of plant survival per pot, according to the mean separation. Therefore they can be categorized as salinity susceptible rice varieties throughout the growing period after seedling stage. Rest of the varieties namely Bg 379-2, Bg 450, Bg 403, Bg 406, Bg 94-1, At 353, Bg 358, Bg 359, Bg 366 and Bg 4-91 can be categorized as moderately salinity tolerant or moderately salinity susceptible varieties.

Varieties in the tolerant group were identified based on percentage of plant survival throughout the growing period after seedling stage. They were analyzed further based on the influence of salinity on PH, PL, RDW, SDW, PWT, PNO, and YLD at 12 dS/m salinity level. Clustering was done at standardized distance of 0.9 in the dendrogram. Two distinct clusters were identified as shown in Figure 2. As a result *Pokkali*was identified as highly salinity tolerant variety whereas Bg 369, At 354 and At 402 were identified as salinity tolerant varieties throughout the growing period after seedling stage.

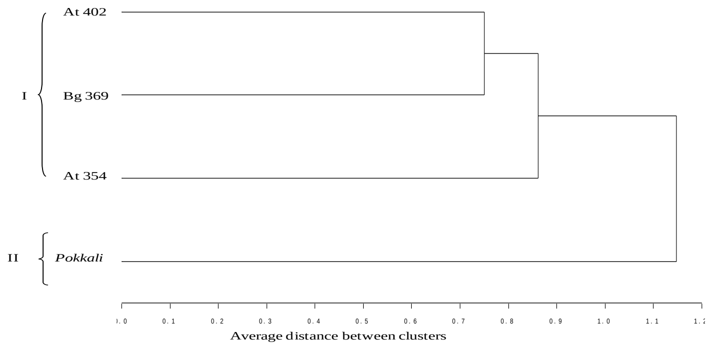

Figure 2. Separation of the varieties in the salinity tolerant group based on % plant survival at a salinity level of 12 dS/m

Moderately tolerant or moderately susceptible groups were clearly identified at 8 ds/m level salinity level based on percentage of plant survival. The data were analyzed further based on the influence of salinity on PH, PL, RDW, SDW, PWT, PNO, and YLD at 8 dS/m salinity level. Clustering was done at standardized distance of 0.75 in the dendrogram. Two distinct clusters were identified as shown in Figure 3. According to the results, Bg 406 was identified as a moderately tolerant variety and Bg 94-1, Bg 4-91, Bg 358, Bg 450, Bg 359, At 353, Bg 366, Bg 403 and Bg 379-2 were identified as moderately susceptible varieties.

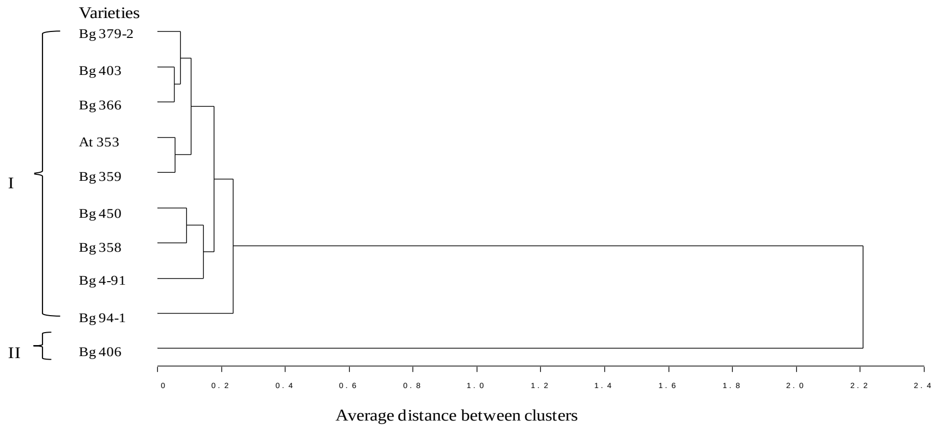

Figure 3. Separation of the varieties in the moderately salinity tolerant or moderately susceptible groups based on % plant survival at a salinity level of 8 dS/m

Rest of the varieties identified as salinity susceptible varieties were further analyzed by using dendogram. Clustering was done at standardize distance of 0.6 in the dendrogram for PH, PL, RDW, SDW, PWT, PNO, and YLD at 4 dS/m salinity level. Two distinct clusters were identified (Figure 4). Bg 300 was identified as a susceptible variety and Bg 362, Bg 352, Bg 365, Bg 357, Bg 307, Bg 308 and Bg 360 were identified as highly susceptible varieties throughout the growing period after seedling stage.

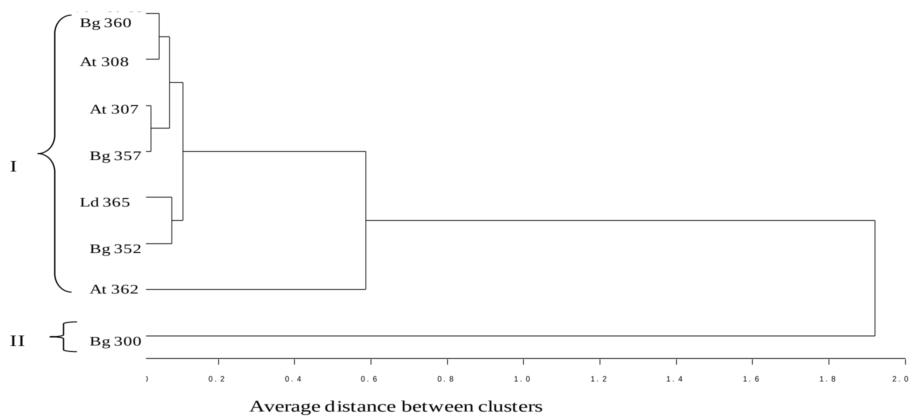

Figure4. Separation of the varieties in the susceptible group based on % plant survival at a salinity level of 4 dS/m

CONCLUSIONS

At seed germination stage, Bg 406 was identified as highly salt tolerant variety whereas *Pokkali* was identified as salt tolerant variety. Further Bg 450 was identified as a moderately salt tolerant at seed germination stage and all the other varieties are susceptible to salinity. At 402 was identified as highly salinity tolerant variety whereas *Pokkali*, Bg 369 and At 354 were identified as salt tolerant varieties at seedling stage. The varieties At 353, Bg 379-2, Bg 403 and Bg 406 were identified as moderately salt tolerant at seeding stage. After seedling stage, 8 dS/m salinity level was identified as the critical salinity level for the screened varieties. Percentage reduction increased with increasing salt levels for PH, PL, RDW, SDW, PWT, PNO and YLD. Based on the phenotypic observations *Pokkali* was identified as a highly salinity tolerant variety whereas Bg 369, At 354 and At 402 were identified as salinity tolerant variety after seedling stage while Bg 406 was identified as a moderately tolerant variety and all the other varieties were salinity susceptible.

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