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Identification of Salt Tolerant Rice Varieties at the Seed Germination Stage and its Relationship to Seed Husk Thickness and Ion Absorption

A. Subasinghe, N.A.A.S.P. Nissanka[1] and W.M.W. Weerakoon[2]

Postgraduate Institute of Agriculture
University of Peradeniya
Peradeniya, Sri Lanka

ABSTRACT. *Salinity is one of the major abiotic stresses which limit the rice production in coastal areas and inland, where irrigated agriculture is practiced. Inland salinity in rice fields is increasing, and if corrective measures are not taken, rice production could be greatly influenced. Therefore, identification of saline tolerant rice varieties and further development of tolerance is the most viable solution. Therefore, this study was carried out to screen salinity tolerant rice varieties at seed germination stages and to study seed characteristics associated with germination under high saline conditions.*

Seeds of several traditional, old-improved, new-improved and hybrid rice varieties were soaked in four different saline solutions (0, 3, 25 and 45 dS/m) for nine days and germination was recorded six days later when seeds were transferred from the solution. Absorption of water, sodium and chlorine by the seeds was analyzed after soaking the seeds in saline water. Seed husk thickness and husk density were also measured. Tested varieties could be categorized into four distinct groups according to its germination ability and seedling survival rate. Pokkali, at 354, Nona Bokra and at 401 could be categorized as the tolerant varieties while Pokkali recorded the highest germination under the highest salinity level. A clear relationship was observed between seed sodium absorption percentage and seed germination of varieties. Seed-husk thickness had affected the Sodium absorption to the seeds and it determined the germination ability of the variety. Therefore, pre-soaking of rice varieties and evaluating subsequent germination could be used as a viable screening method for salinity tolerance at the seed germination level.

INTRODUCTION

Yield of many crop species is reduced by exposure to salt which may be caused either by sea water inundation or by salinization due to high evapotranspiration. In paddy soils, at electrical conductivities (EC) saturated paste below 4 dS/m, rice growth is normal. However, yields of rice may be reduced by 50% or even more at the soil solution EC of 6 - 10 dS/m and beyond 10 dS/m rice yield is drastically reduced (Akbar and Senadhira, 1985). Out of 95 million ha of saline soils in south and South East Asia, about 27 million ha are coastal saline soils (Akbar and Ponnamperuma, 1982).

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1 Department of Crop Science, Faculty of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka.

2 Rice Research and Development Institute, Batalagoda, Ibbagamuwa, Sri Lanka.

During pre-monsoon rainy season, seedlings in nursery beds suffer severely from salinity nevertheless traditional rice genotypes are mostly grown once a year in coastal saline soils taking advantage of the rainy season when salinity levels drop below the critical limit of 4 dS/m in Sri Lanka (Akbar and Ponnamperuma, 1982). The large extent of rice lands (150,000 ha) in the coastal areas as well as in major irrigation schemes in the island have become unproductive due to high salinity (Handawela, 1982).

Reclamation of these lands using chemical compounds is expensive and needs large quantities of water. Therefore, introduction of rice varieties tolerant to salinity is one of the viable solutions towards increasing productivity of these lands (Sirisena and Abeysiriwardena, 2005). There are considerable efforts from plant breeders to select salt tolerant genotypes. Early identification of salt tolerance in breeding program is of great importance but the testing of new genotypes in agronomic trials under saline conditions requires large amounts of seed and can only therefore be undertaken at later stages of evaluation programs (Alam *et al*., 2006). There are two screening methods adopted in Sri Lanka. One is the screening of rice varieties under salt induced production plots while the other method is the screening rice varieties under greenhouse conditions for visual salt injury symptoms (DOA, 1987).

Screening under field and greenhouse conditions is difficult due to stress heterogeneity, presence of other soil-related stresses and the significant influence of environmental factors such as temperature, relative humidity and solar radiation. Germination is a crucial stage in the life of many plants and salt tolerance during this phase is critical to the establishment of plants in saline soils (Maranon *et al*., 1989). It has been reported that germination of several halophytic and glycophytic species was inhibited by salinity (Marcar, 1987). Laboratory investigations of seed germination indicated that seeds of most halophytes and glycophytes attain their maximum germination in distilled water; however, glycophytes are very sensitive to elevated salinity at the germination and during the early seedling phase of development (Norlyn and Epstein, 1984). It was reported that rice varieties capable of germinating after pre-soaking their seeds in saline water for several days were having better seedling survival rate (Abeysiriwardene, 2004, Sirisena and Abeysiriwardena, 2005). However, mechanisms responsible for varietal variation in seed germination and seedling establishments of different rice varieties under saline conditions are not understood. Therefore, this study was initiated to investigate variation of seed characteristics of different rice varieties, salt accumulation pattern in seeds and their influence on seed germination and seedling establishment when they were pre-soaked in saline water.

MATERIALS AND METHODS

Screening was carried out using 16 improved and 10 traditional rice (*Oryza sativa* L.) varieties, which include some known salinity sensitive and resistance types. Seeds were obtained from the Rice Research and Development Institute, Batalagoda (RRDI) and Plant Genetic Resource Centre (PGRC), Gannoruwa in Sri Lanka. The experiment was conducted in the laboratory at RRDI, Batalagoda and chemical analyses were done in the laboratory at the Department of Crop Science, Faculty of Agriculture, University of Peradeniya.

A two factor factorial experiment, factors being the cultivar and salinity, was laid out in a completely randomized design with three replications. Sodium chloride solutions having four electrical conductivity (EC) levels, 0, 3, 25, 45 dS/m were prepared to represent different salinity levels. Each replicate consisted with 100 seeds from each variety which were pre-soaked in these solutions for nine days. After soaking for nine days, seeds were taken out and washed thoroughly to remove the film of salt solution around the seeds. Then seeds were kept in Petri-dishes lined at the bottom with pieces of wet blotting papers. After six days, number of germinated seeds, abnormally germinated seeds and non-germinated seeds were counted. If the seeds had both radicle and coleoptile at the time of six days, germination was considered as normal and other exceptions were considered as abnormal.

A separate sample from selected varieties (representative varieties including salt tolerant, moderately tolerant, sensitive and very sensitive) was taken for sodium and chloride absorption analysis after pre-soaking in saline water and subsequent washing to remove surface salts. Absorption of sodium and chlorine by seeds was analyzed after careful dehusking of seeds. The Sodium (Na^+) content of both grains and husk were analyzed using flame photometer and chloride (Cl^-) content was analyzed in rice grains following the method proposed by Yoshida *et al*. (1971). Individual water absorption of seeds of different varieties after nine days soaking time in saline water (45 dS/m) was calculated using four grams of seeds from each variety.

Thickness of seed-husk was measured using the vernier caliper on the flat side of the seed while preventing ridges being in contact with surface of the vernier. Varieties were categorized for salinity tolerance by their germination ability under saline solutions. Germination percentages of different varieties were analyzed using logistic procedure under categorical data using SAS. Respective correlations between variables were calculated using linear regression procedure using SAS.

RESULTS AND DISCUSSION

Germination at different salinity levels

Germination was reduced across all varieties when the seed were soaked in saline solutions. The greatest reduction was observed in the seeds soaked in 25 dS/m and 45 dS/m saline solutions. The germination was not significantly different at 0 dS/m and 3 dS/m saline conditions (Figure1). The germination percentage of tested varieties was significantly different when the seeds were soaked in 45 dS/m saline solutions (P=0.001). Varieties could be categorized in to four distinct groups based on the percentage seed germination at 45 dS/m as >50% (tolerant), 50 - 20% (moderately tolerant), 20 - 5% (sensitive) and <5% (very sensitive). Interestingly, the most salt tolerant variety, Pokkali recorded the highest germination percentage among the tested varieties and it was as high as 86% on average, whereas At 354 and At 401 and Nona Bokra had germination percentages of 29, 21 and 20%, respectively (Table 1).

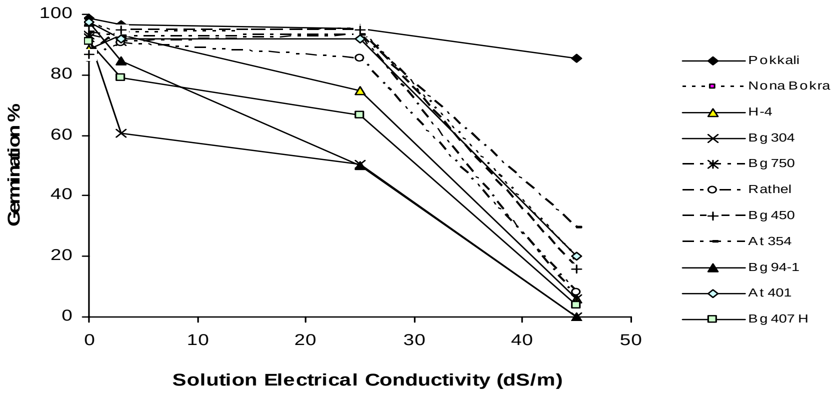

Figure 1. Variation in germination of rice varieties under different saline conditions
Note: Only few representative varieties from sensitive and very sensitive groups are shown in this figure.

Based on the proposed varietal grouping, the variety Pokkali is categorized as tolerant to salinity. Moderately tolerant group includes At 354, At 401 and Nona Bokra and the sensitive group includes H10, Rathel, H4, Bg 750, At 353, Bg 450, Seeraga Samba, Bandara Heththewa, Bg 407 H, Bw 302, Bw 78 and At 303. Varieties recorded the least germination were grouped as very sensitive to salinity which includes Bg 300, Pachchaperumal, Bg 304, Bg 94-1, Periyakarappan, Dikwee, Bg 380, Bg 350, Hondarawalu and Murungakayan. Varietal ranking for salinity tolerance observed in this experiment is in agreement with the finding of Abeysiriwardena (2004) who had ranked Pokkali as highly tolerant and At 354, Nona Bokra and At 401 as tolerant and Bg 450 as moderately susceptible. The susceptible group of Abeysiriwardena (2004) included Bg 94-1, Bg 300, Bg 403 and Bg 350. Response of germination to salinity varies among rice varieties and decreased as the duration of pre-soaking in saline water increased (Abeysiriwardena, 2004). A similar pattern was also observed by Sirisena and Abeysiriwardena (2005) but they have ranked Bg 94-1 as moderately tolerant though it had very low germination after pre-soaking at 45 dS/m of EC. This might be due to differences in pre soaking of seeds in their experiment which was nine days pre-soaking in saline water followed by two day soaking in de-ionized water whereas in this experiment, seeds were pre-soaked for nine days only in saline water. Results of germination were also consistent with Dissanayaka and Wijeratna (2006) who used 21 dS/m saline solution to screen the salinity tolerant rice varieties.

Regulation of seed germination by environmental factors is important in both ecological and agricultural contexts. The interaction between environment stresses and endogenous dormancy mechanisms determine whether a particular seed will germinate under given conditions (Bradford *et al*., 1992). Fully imbibed, non dormant seeds can be expected to initiate radicle growth after a lag period related to the temperature. If the water potential of the imbibition-medium is reduced, germination will be delayed or prevented (Hegarty, 1978). Sodium chloride (NaCl) may readily cross the cell membrane into the cytoplasm of the cell. Entering ions decrease seed osmotic potential which facilitates the hydration of the seed (Katembe *et al*., 1998). Rates of water uptake by rice seed is reduced with increasing salinity. Rice seeds attained full imbibitions in 48 hrs at 150 mM salinity and at 250 mM salinity, seeds reached the critical moisture content by 72 hrs (Alam *et al*., 2003). This suggests that increasing salinity delays the time to attain critical moisture content of rice seeds and thereby the onset of germination. The critical moisture content for germination of rice seeds is around 25 - 30% and it may differ among varieties and with different seed sizes and husk characteristics (Alam *et al*., 2003).

Table 1. Germination percentage of different rice varieties at electrical conductivity of 0 and 45 dS/m.

Variety	Germination (%)		Reduction of Germination (%)	Sensitivity to salinity
	0 dS/m	45 ds/m	at 45 dS/m	
Pokkali	99 ± 1.1	86 ± 3.2	13	Tolerant
At 354	94 ± 6.0	29 ± 2.1	65	Moderately Tolerant
Nona Bokra	94 ± 1.0	20 ± 2.1	74	Moderately Tolerant
At 401	97 ± 2.3	21 ± 1.0	76	Moderately Tolerant
H-10	97 ± 2.3	10 ± 1.0	87	Sensitive
Rathel	91 ± 2.0	8 ± 1.5	83	Sensitive
Seeraga Samba	95 ± 2.1	7 ± 2.0	86	Sensitive
Bandara Heththawa	94 ± 1.5	7 ± 2.0	87	Sensitive
Bg 407 H	95 ± 1.5	8 ± 1.5	87	Sensitive
Bw 302	95 ± 3.0	10 ± 2.0	85	Sensitive
Bw 78	94 ± 2.1	11 ± 3.1	83	Sensitive
H-4	93 ± 4.5	6 ± 3.5	87	Sensitive
Bg 750	93 ± 2.1	6 ± 2.0	87	Sensitive
Bg 450	93 ± 2.6	6 ± 4.0	87	Sensitive
At 353	97 ± 1.0	6 ± 2.5	89	Sensitive
At 303	96 ± 2.0	6 ± 2	88	Sensitive
Murungakayan	99 ± 1.0	3 ± 1.1	96	Very sensitive
Hondarawalu	96 ± 3.1	4 ± 1.5	92	Very sensitive
Bg 380	93 ± 2.3	3 ± 1.0	90	Very sensitive
Bg 350	99 ± 1.0	1 ± 1.0	98	Very sensitive
Pachchaperumal	94 ± 1.5	2 ± 1.5	92	Very sensitive
Bg 300	95 ± 2.3	2 ± 2	93	Very sensitive
Bg 304	92 ± 1.5	0	92	Very sensitive
Periyakarappan	95 ± 3.1	0	95	Very sensitive
Bg 94-1	97 ± 4.6	0	97	Very sensitive
Dikwee	98 ± 2.5	0	98	Very sensitive

Note: Grouping of varieties were based on the sensitivity to salinity.

Variation in water absorption and seed germination at high salinity level

Seeds of all the varieties tested had absorbed higher amount of water when soaked in distilled water compared to saline water (45 dS/m). In distilled water, all the varieties had absorbed 30% - 40% water. The variety Pokkali absorbed the highest water content (30.3%) when soaked in the high saline solution while variety Dikwee recorded the lowest absorption (Table 2). Though, seeds of all varieties absorbed water more than their critical level (25% -

30%) for germination, clear differences in germination were observed among varieties which may be due to toxicity effects of absorbed ions.

There is no clear relationship among varieties between water absorption by individual seeds after nine days of soaking in saline water and their germination percentage (r = 0.45). Lack of such relationship could be partly due to absorption of water above critical level by seeds of all varieties. Some varieties like Pokkali recorded 30.3% water absorption and 86% germination whereas at 354 and At 353 recorded 29% and 6% germination respectively, while having 29.8% water absorption (Table 2).

Table 2. Water absorption by individual seeds of different varieties after seed soaking in pure (0 dS/m) and saline water (45 dS/m).

Variety	Water absorption (%) compare to initial dry weight		Reduction in water absorption (%) at 45 dS/m	Germination(%) at 45 dS/m
	0 dS/m*	45 dS/m*		
Dikwee	31.7^{g}	26.2^{e}	5.5	0
Bg 94-1	36.4^{e}	29.8^{b}	6.6	0
Bg 300	37.8^{bcd}	28.6^{d}	9.2	0
Bg 380	37.9^{cbd}	28.7^{d}	9.2	3
Bg 450	34.8^{f}	29.4^{c}	5.4	6
At 353	38.2^{b}	29.8^{b}	8.4	6
H-4	38.3^{ced}	28.7^{d}	9.6	6
Nona Bokra	38.0^{cb}	28.6^{d}	9.4	20
At 401	36.8^{ed}	29.8^{b}	7.0	21
At 354	37.8^{cbd}	29.8^{b}	8.0	29
Pokkali	39.5^{a}	30.3^{a}	9.2	86

Note: *significant at P = 0.05.
Any means followed by the same letter are not significantly different at the P = 0.05.

Accumulation of Sodium and chloride ions in seeds

All the varieties absorbed more sodium ions when seeds were soaked in saline water. Sodium absorption between varieties was significantly different in 45 dS/m EC solutions (P< 0.05). Some varieties such as Bg 450 recoded 300 ppm more salt compared to Pokkali and Nona Bokra (Table 3). The seed germination was significantly reduced when more sodium was accumulated in seeds. Correlation analysis revealed that there was a stronger relationship among varieties between percentage increases in sodium absorption (%

of Na at 45 dS/m compared to Na at 0 dS/m) and germination percentage at 45 dS/m ($P<0.05$, $r = 0.74$) (Figure 2).

Chloride accumulation was also increased when the seeds were soaked in high salinity levels and showed a significant variation among varieties (Table 3). However, there was no clear relationship between the amount of chloride absorption in to seeds and the germination percentage ($P>0.05$, $r = 0.26$).

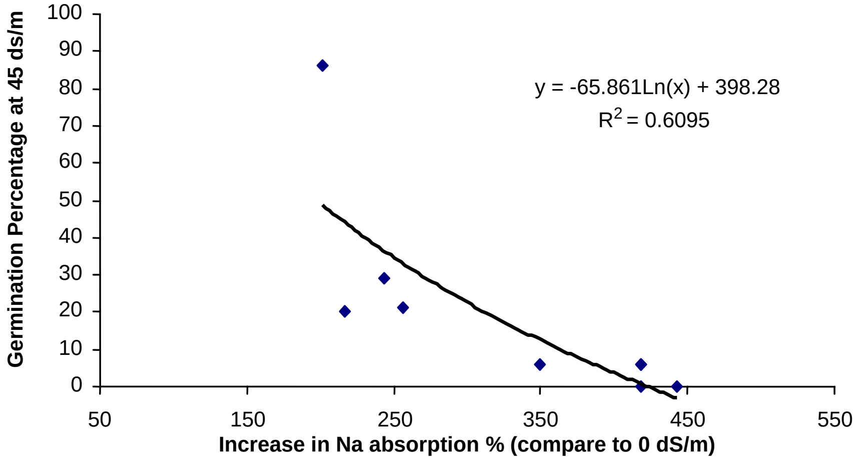

Figure 2. **Germination response of different rice varieties (at 45 dS/m) to percentage increase in Na absorption at 45 dS/m (compare to 0 dS/m).**

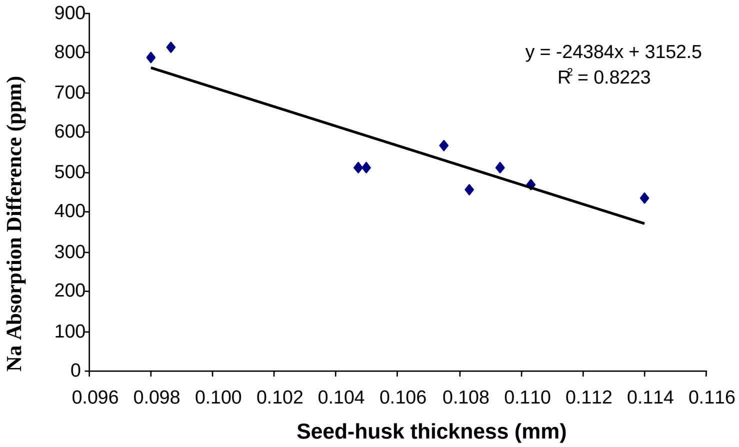

Figure 3. **Relationship between Sodium absorption (ppm) by rice varieties (between 45 dS/m and 0 dS/m) and seed-husk thickness.**

Table 3. Sodium and Chloride concentration and percentage increase over pure water of de-husked rice grain after seed soaking whole grain in saline water (45 dS/m) for nine days.

Variety	Na^+ absorption(ppm)		Increase of Na^+ at 45 dS/m(%)	Cl^- Absorption (%)		Increase of Cl^- at 45 dS/m(%)
	0 dS/m*	45 dS/m*		0 dS/m*	45 dS/m*	
Pokkali	233.0[a]	703.0[d]	201.7	0.35[b]	0.41[c]	16.3
Nona Bokra	200.0[a]	633.3[d]	216.7	0.39[b]	0.42[c]	9.1
At 354	233.3[a]	800.0[c]	242.9	0.37[ab]	0.42[c]	14.3
At 401	177.8[b]	633.3[d]	256.2	0.32[c]	0.44[b]	38.3
Bg 380	177.8[b]	800.0[d]	349.9	0.50[a]	0.64[a]	28.6
Bg 450	233.3[a]	1050.0[a]	350.1	0.28[ef]	0.32[g]	12.5
At 353	122.2[b]	633.3[d]	418.2	0.27[f]	0.35[e]	33.3
Dikwee	122.2[b]	633.3[d]	418.2	0.30[d]	0.34[f]	11.8
H 4	122.2[b]	633.3[d]	418.2	0.28d[e]	0.34[f]	18.8
Bg 300	177.8[b]	966.0[b]	443.3	0.23[g]	0.37[d]	61.5
Bg 94-1	177.8[b]	966.0[b]	443.3	0.35[b]	0.50[h]	40.0

Note: * significant at P = 0.05 probability level.
Any means followed by the same letter are not significantly different at the P = 0.05.

Table 4. Effect of seed size (compared to Rathel) on salinity tolerance of rice varieties of different age classes.

Variety	Seed size*	Sensitivity to salinity	Age Class (months)
Rathel	1.000	Sensitive	4 - 4 1/2
Bg 450	1.033[i]	Sensitive	4 - 4 1/2
Dikwee	1.501[h]	Very sensitive	4 - 4 1/2
Bg 304	1.754 [g]	Very sensitive	3
Pokkali	1.933[f]	Tolerant	3 1/2
Bg 300	1.965[f]	Very sensitive	3
H 10	2.040[e]	Sensitive	3
At 353	2.062[ed]	Sensitive	3 1/2
Bg 94-1	2.093[d]	Very sensitive	3 1/2
At 354	2.142[c]	Moderately Tolerant	3 1/2
At 401	2.187[c]	Moderately Tolerant	4 - 4 1/2
H 4	2.206[b]	Sensitive	4 - 4 1/2
Nona Bokra	2.240[b]	Moderately Tolerant	4 - 4 1/2
Bg 750	2.314[a]	Sensitive	2 - 2 1/2
Pachchaperumal	2.338[a]	Sensitive	3 1/2

Note: significant at P = 0.05, * Seed Size – Seed weight ratio to the smallest seed - Rathel
Any means followed by the same letter are not significantly different at the P = 0.05.

Seed properties and ion absorption and germination

Seed-husk thickness was different among varieties and sodium absorption was reduced with increasing husk thickness (r = 0.90). Higher salt accumulation inside the seeds affected germination of rice varieties (Figure 2).

Individual seed size was significantly different among the varieties tested. However seed size has no relation with the germination ability of a particular variety under saline conditions (Table 4). Age class of a particular variety has no affect on its germination ability under saline conditions (Table 4).

CONCLUSIONS

Pre-soaking of rice seeds using 45 dS/m saline water can be used to screen rice varieties for salinity tolerance at the seed germination level. Nine days pre-soaking of rice seeds in saline water will reduce the water absorption and increase the Sodium and Chloride content in seeds. Sodium is absorbed to the seeds along with imbibitions of water. There are differences in water, Sodium and Chloride absorption among varieties. Reduction in rate of water uptake at high salt solutions had no effect on germination due to its ability to attain critical moisture leveling at nine days of pre soaking. A cumulative effect of these three factors inside the seed might determine the germination ability of a particular rice variety under osmotic stress conditions caused by saline water. Ability of the seed coat in avoiding salt entrance into the seed during imbibitions when soaked in saline water is correlated with husk thickness. These findings suggest that, germination testing after pre-soaking rice seeds in 45 dS/m saline solutions could effectively be used to screen the rice varieties for salinity tolerance.

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