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Effect of Alternating Saline and Non-saline Conditions on Emergence and Seedling Growth of Rice

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Abstract: A pot experiment in glasshouse was conducted with a single salinity level of 10.5 dS m^{-1} to study the effect of alternating saline and non-saline conditions on emergence and seedling growth of two rice cultivars (IR8 and V3) differing in salt tolerance. A split-plot design was used with three replications. The results demonstrated that rice seedlings, 1 to 3 weeks after emergence, proved to be very salt-sensitive. There was about 80% reduction in stand establishment in 10.5 dS m^{-1} salt (about 115 mM) imposed for just one week. Maintaining rice seeds in saline conditions from seeding resulted in 50-70% initial seedling emergence. However, most seedlings then died within 14 days and by 42 days, only 5% or less survived. Though substantial differences existed between cultivars during germination and emergence, cultivar differences during post-emergence growth were minimal. Little improvement in stand establishment and seedling growth was achieved by ameliorating an initial exposure to salt by transferring to water after 21-35 days. The results demonstrated a gradual decrease in seedling mortality with later exposure to salt. There was little effect on stand establishment and other plant growth parameters when saline solution was introduced 35 days from seeding. Tolerance to a given salt concentration during germination and emergence of rice was not accompanied by the plants tolerance to that particular salt concentration at later stages of growth.

Key words: Salinity, rice, seedling growth, seedling emergence

INTRODUCTION

Salinity has been an important historical factor influencing the life spans of agricultural systems and destroying ancient agrarian societies[1]. Despite the advanced management technologies available today, salinization of millions of hectares of land continues to reduce crop production severely worldwide. Since a technological improvement in the situation is improbable in near future[2] traditional means of ameliorating salt affected soils through reclamation, drainage and use of excess irrigation water to leach salts below the root zone have to be complemented with a biological approach to the problem. Sustained and profitable production of crops on salt-affected soil is possible if appropriate farm management decisions are made. To be successful, growers require an understanding of how plants respond to salinity, the relative tolerance of different crop cultivars and their sensitivity at different stages of growth to soil and environmental conditions.

The tolerance to salinity of a given species or cultivar may change during ontology. It may decrease or increase, depending on the plant species, cultivar or environmental factors. Sugar beet, for example, is highly tolerant during most of its life cycle but sensitive during germination. In maize, salt sensitivity is particularly high at tasseling and low at grain filling[3]. In contrast, rice is moderately susceptible to salinity, since most rice plants are severely injured at an EC of 8-10 dS m^{-1}. Below an EC of 4, rice growth is normal[4]. Sensitivity of rice to salinity varies greatly with growth stage. Rice is very tolerant during germination but very sensitive at the early seedling stage. Its salt tolerance progressively increases during tillering and elongation and decreases at flowering. Ripening appears to be little affected by salinity[5]. A considerable degree of varietal difference in salinity tolerance in rice also appears to exist.

Because of the differential plant responses under saline conditions, a package of production technologies including consideration of the appropriate age of seedlings for transplanting, the time of planting, recovery after salt shock etc. are necessary for successful growth of rice in saline conditions in the field. This study was initiated to identify the relatively more or relatively less salt-sensitive or salt-tolerant growth stages of rice seedlings with a view to identifying the optimum growth stage of rice seedlings for transplanting. The other objective was to study the recovery of rice seedlings from salt shock at different growth stages.

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MATERIALS AND METHODS

IR8 and a salt-tolerant[6] advanced breeding line BR5331-93-2-8-4 (denoted here as V3) were chosen (as they were phenotypically very similar) as the experimental plants to represent salt-sensitive and salt-tolerant responses, respectively in a pot experiment in the glasshouse. A split-plot design was used with three replications. Twelve rice seeds were sown in each 12.5 cm square x 11.5 cm plastic pot containing 1.5 kg of air-dry soil. The pots were then arranged (partially submerged in saline water) in trays (45 x 25 x 10 cm). Each tray contained two pots of each of V3 and IR8. The salt treatments were applied to the trays. Each tray was considered as main plot and each pot/cultivar was considered as a sub-plot. After emergence, 9-10 plants were maintained in each pot. Salt was applied at a single salinity level and the conductivity in the trays was maintained at 10.5 dS m^{-1} (about 115 mM). Exposure to salt was for a single week. The treatments were thus;

T_1= Salt condition throughout
T_2= Salt wk. 1, non-salt wk. 2-6
T_3= Non-salt wk. 1, salt wk. 2, non-salt wk. 3-6
T_4= Non-salt wk. 1-2, salt wk. 3, non-salt wk. 4-6
T_5= Non-salt wk.1-3, salt wk. 4, non-salt wk. 5-6
T_6= Non-salt wk. 1-4, salt wk. 5, non-salt wk. 6
T_7= Non-salt condition throughout

Treatment 0-d	7	14	21	28	35	42
T_1	Salt conditions throughout					
T_2	Salt					
T_3		Salt				
T_4			Salt			
T_5				Salt		
T_6					Salt	
T_7	No-salt conditions throughout					

Shading indicates duration of salt imposition

When changes were made from salt conditions to non-salt conditions, pots were supplied with fresh water. The water was changed with further fresh water every morning for the next week to facilitate removal of any remaining salt. Plant height, leaf number per plant and stand establishment were counted at 14, 21, 28, 35 and 42 days after seeding (DAS). Shoot fresh weight, root fresh weight, shoot dry weight, root dry weight and leaf area were measured at 42 DAS. Shoot height was measured from the root base to the tip of the longest leaf, excluding any dead portion. Root lengths were measured from the root-shoot junction to the tip of the longest root. Leaf area (green portion only) was measured by a leaf area meter (model MK2, DELTA-T DEVICES, Burwell, England). Plant samples were dried at 70°C for 72 h for dry weight measurements.

Mean emergence time (MET) was calculated as; $MET=\sum nd/N$ (days) (where n is the number of emerged seedlings on each day, d is the number of days from the beginning of the test and N is the total number of seedlings). Emergence rate (ER) was calculated as; $ER=1/MET$ (seedlings/day).

The data were analysed using standard statistical procedures in GENSTAT. The statistical significance of differences between pairs of treatments was determined by Student's t-test. All percentage data were transformed to arcsine values before statistical analysis.

RESULTS

Seedling emergence: Percent seedling emergence (recorded 14 days after seeding) did not differ significantly in any treatment except in T_1 and T_2 (Fig. 1). However, emergence in T_1 and T_2 differed between the two cultivars. In the salt-tolerant cultivar V3, seedling emergences were about 75 and 85% relative to non-salt stressed plants (T_7) in T_1 and T_2, respectively whereas in the salt-sensitive cultivar IR8, seedling emergences were about 55% in both treatments. In IR8 however, some of seedlings in T_2 continued to emerge after 14 days from seeding.

Emergence rate (ER): The emergence rate of V3 was significantly higher than IR8 regardless of the treatment imposed (Fig. 2). Like seedling emergence, ER in any of the treatments did not differ significantly except in T_1 and T_2 (while considering cultivars individually) in both the cultivars and there were differences between the cultivars. In the salt-tolerant cultivar V3, ER was reduced by about 20% in T_1 and T_2 whereas, in the salt-sensitive cultivar IR8, RE was reduced by about 25% relative to untreated plants (T_7).

Stand establishment: In all cases, including the untreated control (T_7), the number of plants decreased with time (Fig 3a and b). When plants were continuously treated (T_1) with salt (115 mM), all plants died within 28 days of the start of the experiment in the proposed salt-tolerant cultivar V3. In contrast surprisingly, about 10% seedlings survived beyond 42 days (when the plants were harvested) in the salt-sensitive cultivar IR8. In treatments T_3 to T_5, early application of salt caused greater loss of plants in V3. In T_6, measurements were made up to 7 days after the end of the treatment but at this time more plants

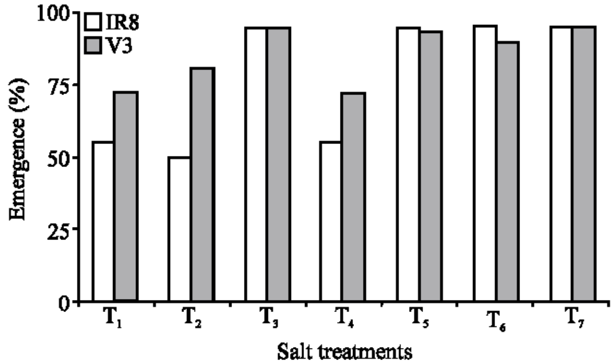

Fig. 1: Effect of different salt treatments on emergence of R8 and V3 at 14 days after seeding (DAS)

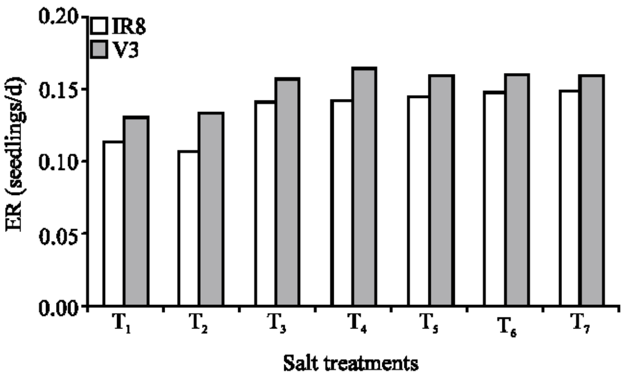

Fig. 2: Effect of different salt treatments of emergence rate of IR8 and V3 at 14 DAS

survived than in the untreated control (T_7). In the case of IR8, similar trends were seen. However, more plants survived in treatment T_2 than T_3 in IR8.

Plant height: Plant height in all the treatments increased with time in both the cultivars except V3 in T_1 (as all plants died in this treatment after 21 days). However, there were significant differences in growth rates between treatments and between cultivars as well (Fig. 4a and b). At 42 days after seeding (DAS), plant heights in all the treatments were significantly shorter than untreated plants except T_6, with the greatest effect in T_3 for both the cultivars. At this growth stage, plant height in T_6 was very similar to that of untreated plants (T_7) of both the cultivars. The rate of increase in plant height was greatest in T_2 and least in T_3 in both the cultivars.

Leaf number: Early application of salt almost completely inhibited leaf production in T_1, T_3 and T_4 (but not in T_2) in both the cultivars. Leaf numbers in all other treatments increased with time although, with a reduced rate in T_2 (Fig. 5a and b). At 42 DAS leaf numbers in T_5 and T_6 were similar to those of untreated plants (T_7) in both the cultivars.

Leaf area (LA): Significant differences in LA at 42 DAS were observed between V3 and IR8 even in the absence of salt (about 42 and 38 cm^2 $plant^{-1}$, respectively). Leaf area in T_6 was very similar to that of T_7 in both the cultivars. Leaf areas in all other treatments were significantly lower than untreated plants with the largest effect in T_1. In treatments T_2 to T_3, the reductions in LAs were very similar in both the cultivars. However, in T_6 the reduction in LA compared to untreated plants was about 55% in V3 whereas it was only about 20% in IR8 (Fig. 6).

Root length: Root length was less affected by salt treatments compared to LA. In treatments T_1 to T_4, early imposition of salt caused significant reductions in root lengths with relatively higher effects on T_1 and T_3. Root lengths in T_5 and T_6 were very similar to those of untreated plants (T_7) in both the cultivars (Fig. 7).

Shoot fresh weight (SFW): Reduced SFWs were observed in all the treatments except in T_6 and T_7 in both cultivars, with greater effects in T_1 and T_3. However, in any particular treatment, SFW of the sensitive cultivar IR8 was significantly higher than that of the tolerant cultivar V3 (Fig. 8).

Shoot dry weight (SDW): Shoot dry weights in T_2, T_5 and T_6 were very similar to those of untreated plants in both the cultivars. Significant reductions in SDWs were recorded in other treatments compared to untreated plants with greater effects in T_1 and T_3 (Fig. 9).

Root fresh weight: Root fresh weights in T_2, T_4 and T_6 were little or not affected at all by the salt treatments in both the cultivars. Significant reductions in RFWs were observed in all other treatments with higher effects in T_1 and T_3 (Fig. 10).

Root dry weight (RDW): Significant cultivar differences at 42 DAS were observed in RDWs following imposition of the salt treatment (Fig. 11). Like RFW, RDWs in T_2, T_4 and T_6 were very similar to those of untreated plants (T_7) in IR8. In contrast, RDWs in T_4 and T_5 were similar to those of T_7 in V3 and in T_2 and T_6 were about 35 and 10% higher than T_7, respectively. In IR8, root dry weight in T_1 was only about 10% relative to untreated plants, while it was similar (about 70%) in T_3 and T_5 compared to untreated plants. Root dry weight in T_3 was about 65% relative to untreated plants in V3.

DISCUSSION

The results of this study show that rice is substantially tolerant to salt during germination and seedling emergence. However, there were pronounced differences between cultivars in sensitivity to salt during post-emergence growth. When rice seeds and seedlings were maintained in saline solution (10.5 dS m^{-1} = about 115 mM) through out reductions in seedling emergences were about 30% in the tolerant cultivar V3 and about 50% in the sensitive cultivar IR8 (Fig. 1). However, most seedlings died within 14 days and by 42 days only 5% or less survived in all cultivars. This indicated that although substantial differences exist between cultivars during germination and seedling emergence, cultivar differences during post-emergence growth were smaller. Khan *et al.*[7] reported that after 3 weeks of 200 mM NaCl imposition, only three cultivars out of nine survived and those were Pokkali, Kalobail (local varieties) and IPK 37011.

Imposition of salt at 7 days after seeding (DAS) for just 1 week caused about 70-80% reduction in stand establishment and the reduction was greater in the proposed tolerant cultivar V3, than in the sensitive cultivar IR8 (Fig. 3a and b) indicating that the post-emergence early leaf development stage was a critical period for rice growth under salt stress. Shifting the time of salt application to 14 or 21 DAS also for the same period of 1 week gradually reduced seedling mortality with greater improvement in the sensitive cultivar IR8. This indicated tolerance to a given salt concentration during seed germination and emergence of rice is not always accompanied by the plants tolerance to that particular salt concentration at later stages of growth. In both cultivars, introducing saline solution after 35 days had little effect on stand establishment and other plant growth parameters (plant height, leaf number, leaf area, shoot and root weight), indicated salt tolerance of the rice seedling increases with seedling age and around 35-day-old seedlings would better able to withstand salt than younger seedlings. In this study 35-day-old seedlings had 4 to 5 leaves per plant in unstressed plants. Other researchers have also demonstrated negative effects of salt on plant growth and stand establishment and that plants exhibited different sensitivities to salinity at different stages of growth. Pearson and Ayers[8] noted that salinity application to 2-w-old rice seedlings reduced survival and growth. Shannon *et al.*[9] reported that increasing salinity reduced plant growth and stand establishment in young rice plants and rice sensitivity to salt changed noticeably during early development and varied between cultivars. There are similar reports from Khan *et al.*[7] on rice and Rogers *et al.*[10] on white clover.

In all cultivars, ameliorating an initial exposure to saline solution by transferring to water after 21 or 35 days did little to improve stand establishment and seedling growth, again indicating the post-emergence early leaf development stage of rice as the most critical period. However, when seeds were sown in saline solution, but transferred to water after 7 days, the stand establishment improved slightly (about 20%) in the salt-tolerant cultivars V3 but increased greatly (about 60%) in the salt-sensitive cultivar IR8 (Fig. 3). This seemed to be because of the differential tolerance and rate of emergence during germination of these two cultivars under salt conditions.

Due to the salt sensitivity of IR8, there was only about 60% emergence during the first 14 days from seeding and most of these seedlings died subsequently. However, the rest of the seeds remained dormant and emerged when the salt solution was removed after 7 days. This might be termed an 'escape' strategy for coping with short-term salt exposure. In contrast, in the salt tolerant cultivars V3, most seeds emerged within the 7 d period of salt conditions and died as this stage of seedling growth is the most sensitive to salt. Induction of dormancy in IR8 under salt conditions and good recovery under non-salt conditions is supported by our other studies[6]. When salt conditions were maintained throughout, some of the seedlings in IR8 emerged even after 21 days and the seedlings looked apparently healthy, indicating pre-treatment of rice seeds with salt solution might increase salt tolerance especially in salt-sensitive cultivars as has been seen in sorghum and wheat. Following 20 d of exposure to 75 or 150 mM NaCl, sorghum plants became capable of growing in medium containing 300 mM NaCl. Control plants, which were not pre-treated, or plants pre-treated for less than 20 d died within two weeks when exposed to 300 mM NaCl[11]. More recently, Steppuhn and Wall[12] showed that, in the case of wheat, adding salt after emergence resulted in greater salt tolerance than subjecting plants to full salinity at seeding. However, the exact physiological mechanisms involved in the effectiveness of pre-sowing treatments in these observations remain to be elucidated.

This induction of a capacity to survive in and tolerate a high NaCl concentration can be considered as an adaptation to salinity. Adaptation to salinity may be more important than osmotic adjustment because it takes longer time to develop than osmotic adjustment. Adaptation, probably also comprises the development of a capacity to regulate internal Na^+, K^+ and Ca^{2+} concentrations when external salinity is high.

This finding contrasts with the idea that rapid germination/emergence is an important selection criterion for rice cultivars under salt stress in order to obtain better stand establishment and to avoid the risk of

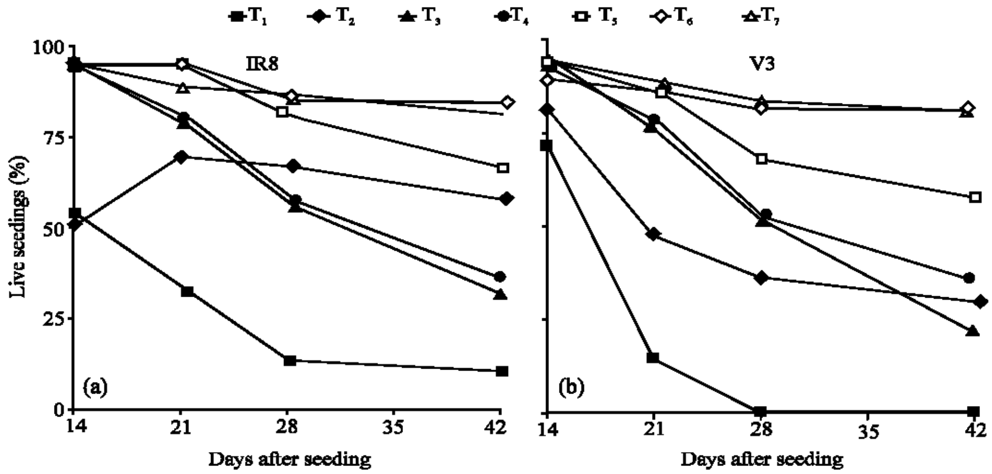

Fig. 3a and b: Effect of different salt treatments on stand establishment or IR8 ans V3 at different DAS

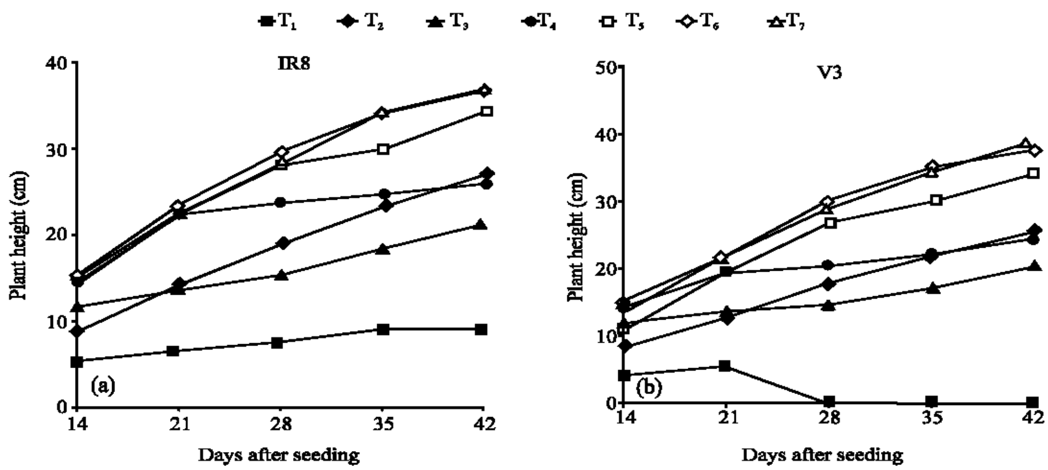

Fig. 4a and b: Effects of different salt treatments on plants height of IR8 and V3 at different DAS

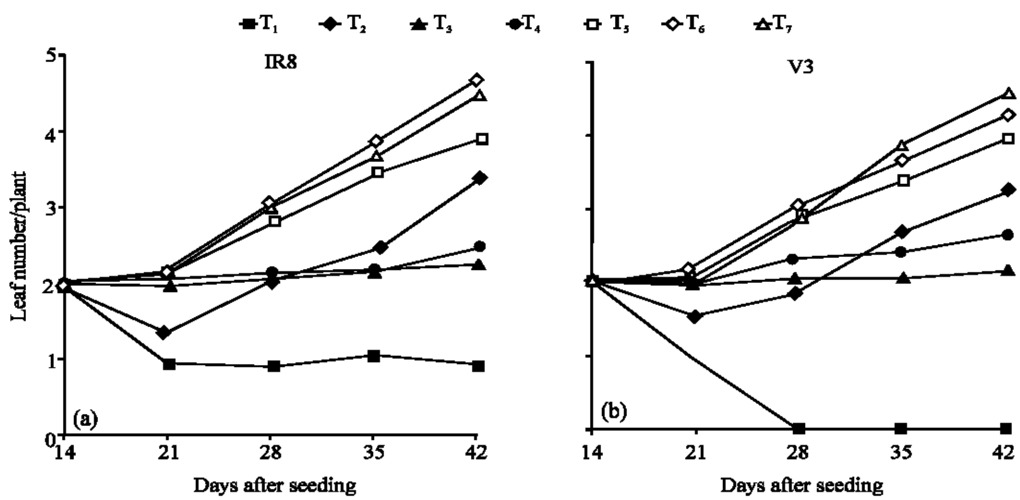

Fig. 5a and b: Effect of different salt treatments on leaf number of IR8 and V3 at different DAS

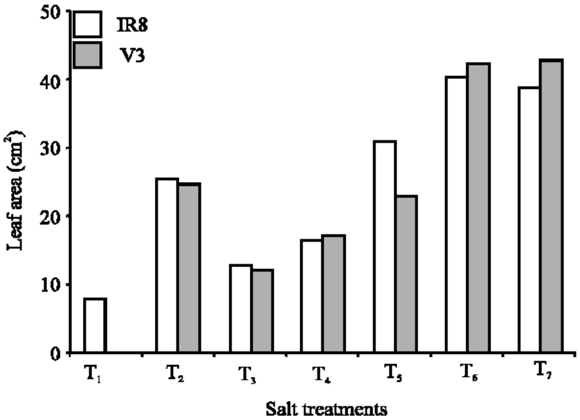

Fig. 6: Effect of different salt treatments on leaf area of IR8 and V3 at 42 DAS

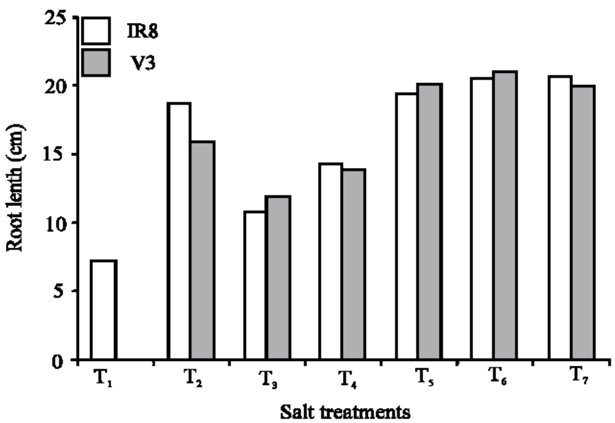

Fig. 7: Effect of different salt treatments on root length of IR8 and V3 at 42 DAS

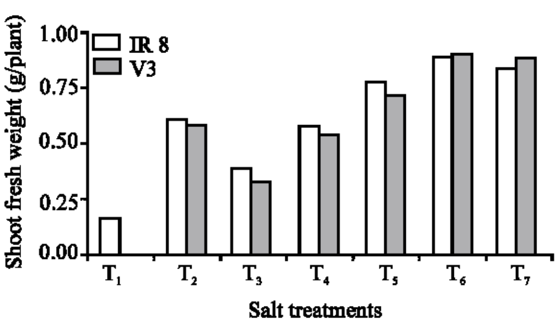

Fig. 8: Effect of different salt treatments on shoot fresh weight of IR8 and V3 at 42 DAS

pre-emergence pest attack. Delayed emergence may also be an important selection criterion for salt-tolerant rice cultivars as it could increase salt tolerance during post-germination growth and give better stand establishment. This is in agreement with the suggestions of Ungar[13] who also reported that tolerance of seeds to

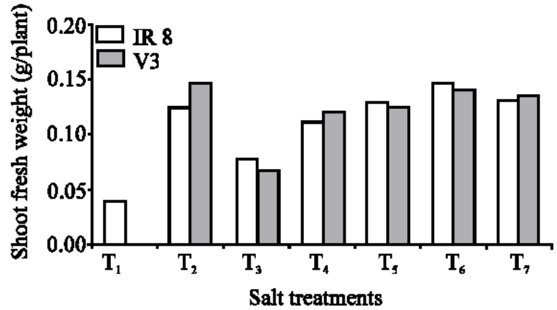

Fig. 9: Effect of different salt treatments on shoot dry weight of IR8 and V3 at 42 DAS

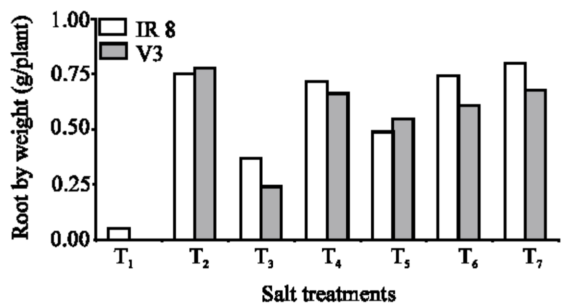

Fig. 10: Effect of different salt treatments on root fresh weight of IR8 and V3 at 42 DAS

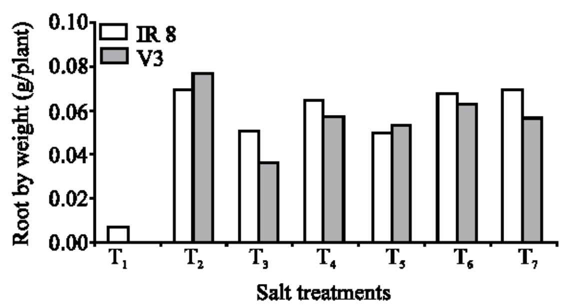

Fig. 11: Effect of different salt treatments on roots dry weight of IR8 and V3 at 42 DAS

salinity should be interpreted at two levels: I) the ability to germinate at high salinitiesand ii) the ability to recover and germinate after exposure to high salinity. Delayed emergence would be advantageous in transient salinitiesand can be considered as salt avoidance or an escape strategy.

One possible explanation for the extreme sensitivity of the post-emergence leaf development stage of rice might be that Na^+ and Cl-are more poorly compartmentalised in young shoot tissues at this stage of growth, because young meristemetic cells lack enough vacuoles to compartment the ions[14]. Catalan *et al.*[15], Rogers *et al.*[10] and Carvajal *et al.*[16] also suggested that plants show NaCl sensitivity during germination and young seedling growth due to poor compartmentation of Na^+ and Cl-ions. Another alternative explanation might be the relative accumulation of K^+ and Ca^{2+} in young shoot tissue. Alam[6] reported that any increase in K^+ and Ca^{2+} influx i.e. improved K^+/Na^+ and Ca^{2+}/Na^+ selectivity, appeared to be related to salt tolerance in rice, as has been seen in cotton[17]. Cramer *et al.*[18] and Suhayda *et al.*[19] also showed that the Ca^{2+} status of the plant is important in salt tolerance. As Ca^{2+} and K^+ accumulate with seedling age, this may in part explain why seedling growth immediately after post-emergence is the most salt sensitive. This consideration merits further work. The notably higher stand establishment and relatively better post-emergence seedling growth of the sensitive cultivar IR8, than the tolerant cultivars V3 was probably associated with larger seed size and higher vigour. This was also shown to be the case by Roundy *et al.*[20] in wild rye and wheat grass and by Rogers *et al.*[10] in white clover. Naylor[21] also showed that germination of both wheat and triticale was greater from bigger seed. Although in triticale, the smaller, lighter seeds also had a lower K_i (an estimate of seed vigour), in wheat the different seed size classes had similar K_i values. It was determined that the seed vigour of IR8 was considerably higher than V3[6]. Larger seeds, which have greater seed reserves, also generally produce more vigorous seedlings, which in turn are more tolerant to NaCl[22,23] perhaps due to a dilution effect of growth conferring lower cellular ion concentrations[24]. This contrasts with our other findings where IR8 performed poorly in germination, plumule and radicle growth and also in mature plant growth stages compared to the salt-tolerant cultivars V3[6]. Such different sensitivities to salinity at different stages of growth are supported by many others[25-28]. Therefore, larger seed size could be a selection criterion for salt tolerant rice cultivars.

The implications of these findings are that direct seeding of rice would not be suitable in saline areas because the young seedlings are very sensitive to salt for a few weeks from emergence. When transplanting rice seedlings into saline areas, using seedlings aged over 35 days would reduce seedling mortality. However, this might limit machine transplanting in saline areas, because young seedlings around 14-d-old (grown in seed trays) are generally used for machine transplanting. Therefore, to select salt-tolerant cultivars of rice will require selection for tolerance at each critical growth stage. The definition of ‘critical growth stage’ will depend on the environment to which plants will be exposed as part of the specific crop management system. For example, if plants are to be transplanted or are to be grown in an area where soil salinity can be kept low during the early stages of growth, then tolerance at germination and during early seedling growth will not be required. This is the case in Bangladesh, during June-July when the incessant rainfall reduces soil salinity to below 4 $dS\ m^{-1}$ and is the planting time of wet season rice. However, if plants are to be direct seeded or mechanically transplanted into moderately saline soils, they must possess salt tolerance at all growth stages. However, the recovery of rice seedlings after transplanting into saline soil needs to be quantified.

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