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Evaluation of Rice (*Oryza sativa* L.) Cultivars Response to Salinity Stress Through Greenhouse Experiment and Tissue Culture Technique

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Abstract: The response to salinity stress of 4 rice cultivars was evaluated through a greenhouse experiment and a tissue culture technique at Agricultural College, Mazandaran University, Sari, Iran, during 2003. In the first experiment, carried out at seedling stage, three salinity levels (0, 75 and 150 mmol NaCl) were used, pots arranged as a split plot based on completely randomized design with four replications and traits including seedling dry weight, wet weight, shoot length and root length were measured. In the second experiment, cultivars were callus induced trough the seed culture and calli placed in LS mediums containing four salinity levels (0, 75, 112.5 and 150 mmol NaCl). The experimental lay-out was factorial based on completely randomized design with 6 replications and callus volume of cultivars was measured according to Hooker and Nabors scale. The results indicated that all cultivars were influenced by increasing salinity level from 0 to 150 mmol NaCl in all traits. Tichung-65 distinguished the most salt-tolerant cultivar, showed the lowest reduction in evaluated traits by increasing salinity stress, whereas Tarom chaloosi was the salt-sensitive one. Moreover, cultivars including Tarom chaloosi and Binam chaloosi, exhibiting low salt tolerance characteristic in seedling stage generated tolerant calli through tissue culture technique as the salinity level increased from 0 to 150 mmol NaCl.

Key words: Salinity stress, rice cultivars, greenhouse experiment, tissue culture technique, salt-tolerant

INTRODUCTION

Plant growth and development are adversely affected by salinity, a major environmental stress that limits agricultural production (Lauchli and Grattan, 2007). Nearly 20% of the world's cultivated area and nearly half of the world's irrigated lands are affected by salinity (Zhu *et al*., 2001).

Rice (*Oryza sativa* L.) is one of the most staple foods in the world the second main food consumed in Iran. It is rated as an especially salt-sensitive crop. The response of rice to salinity varies with growth stages. In the most commonly cultivated rice cultivars, young seedlings were very sensitive to salinity whereas, rice was more salt tolerant at germination than other stages (Zeng and Shannon, 2000). Although the salinity is not considered the most major rice production constraint in Iran, the potential yield of most rice varieties significantly reduces as a result of soil salinity increase in costal regions, the main rice production area in Iran. Therefore, to produce rice in regions affected by salinity problems, an alternative reclamation is to grow salt-tolerant cultivars. But, the improvement and selection of salt-tolerant crops and varieties in the field is not efficient because soil salinity varies substantially with time, location, soil type and depth. Therefore, due to the simplicity, the ease of use and consistent separation of genotypes, greenhouse investigations are developed to evaluate plants based on their relative ability to survive under saline condition. This is an efficient method to evaluate salt tolerance characteristic in different NaCl concentrations based on the survival ability of genotypes. In this regard, determination of salinity-sensitivity parameters, for instance thresholds of salinity effects on rice seedling growth and yield components and the interrelationship among yield components under salinity stress, will help to develop better management practices for growing rice under salinity and improve our understanding of the mechanisms of salt tolerance in crops (Zeng and Shannon, 2000). Besides, considering the fact that all varieties of rice, do not have the same sensitivity to salts, it may not be difficult to find out a salt-tolerant variety or even to develop one through breeding. If proper attention is given towards this direction a salt-tolerant variety of rice may be solution to the adverse situation like coastal areas. Selection of crops for their tolerance is thus, an important aspect for management of saline soils (Gain *et al*., 2004). In this case, the application of tissue culture technique can evaluate crops' tolerance to biotic and abiotic stress factors. The selection of tolerant plants

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to stress factors particularly salinity stress, could be practiced by imposing the stress factor in callus medium to obtain tolerant lines (Vajrabhaya *et al*., 1989). Tissue culture, recognized as a novel means to generate genetic variability, is possibly a new tool for improving salt tolerance in crop species. Several scientists have attempt to increase the salt tolerance of some plant species, both at cellular and the whole plant level by tissue culture techniques. At the moment more knowledge is available at the cellular level than at the whole plant level. Therefore, tissue culture technique is a quick and appropriate method to assess plants' germplasm and to generate salt-tolerant plants (Cano *et al*., 1998). Zair *et al*. (2003) used *in vitro* selection method to isolate salt-tolerant rice cultivars. They found that a useful method for selection of salt tolerant rice cultivars is the regeneration from calli that are grown at high levels of salt concentrations.

The objective of this study which was carried out in two stages was fist to develop a greenhouse experiment that is simple and consistently separates genotypes for their relative ability to survive under saline conditions and second to evaluate the use of tissue culture technique to determine and develop the salt-tolerant rice cultivars. Moreover, the relationship between these two experiments to produce salt-tolerant cultivars is important.

MATERIALS AND METHODS

The experiment was carried out at Agricultural College, Mazandaran University, Sari, Iran, during 2003. This study comprised two stages, a greenhouse experiment and the tissue culture.

Greenhouse experiment: To evaluate the salt tolerance characteristic, a greenhouse experiment was conducted over four rice cultivars including Tarom chaloosi (Local), Binam chaloosi (Local), Hasani (Local) and Tichung-65 using three salinity levels (0, 75 and 150 mmol NaCl). This experiment was conducted as a split plot based on completely randomized design with four replications. The treatments were salinity levels (0, 75 and 150 mmol NaCl) as the main plots and four cultivars (Tarom chaloosi, Binam chaloosi, Hasani and Tichung-65) as the sub plots. First, seeds were disinfected by a 50% Jawex solution. Ten seeds of each cultivar were placed in pots containing perlit, Yoshida solution and different salinity levels and were arranged based on completely randomized design. As the seedling reached 4-leaf stage, plants were carefully taken out from pots, were dried by filter paper and then traits including seedling dry weight, wet weight, shoot length and root length were measured for all treatments. The greenhouse temperature was 25°C with 12 h daylight over this experiment.

Tissue culture experiment: Selected cultivars were also examined through the tissue culture technique at a laboratory. In this stage, four cultivars were evaluated at cellular phase and callus was induced through the seed culture. The experimental lay-out in this stage was factorial based on completely randomized design with six replications. The examined factors comprised four salinity levels (0, 75, 112.5 and 150 mmol NaCl) and four rice cultivars (Tarom chaloosi, Binam chaloosi, Hasani and Tichung-65). First, 10 seeds of each cultivar were sterilized in a 50% Jawex solution containing 2 drops of Twin 20 for 30 min. Then, seeds were rinsed with distilled water and cultured in callus induction medium optimized according to the LS medium (Linsmaier and Skoog, 1965). Cultures were placed in incubator at 26°C and unlighted condition. After twice subculture, as the calli reached 3-4 mm in diameter, they were transferred to petri dishes of salt stress medium comprising different salt concentrations. The callus of each cultivar was placed in each salinity level for 3 weeks and after measuring their volume they were transferred to a higher salinity level. The Trait evaluated in this stage was callus volume difference before and after the salinity stress through the Hooker and Nabors scale (Hooker and Nabors, 1977).

RESULTS AND DISCUSSION

Tolerance at emergence is based on survival, whereas tolerance after emergence is based on decrease in growth or yield (Mass, 1986). The results indicated that traits including seedling dry weight, wet weight, shoot length and root length were influenced ($p<0.01$) by cultivars, salinity levels and also the interaction between cultivars and salinity levels. Increasing salinity level reduced traits mentioned above however, the alteration process over these cultivars is very important as the salinity stress increases from 0 to 150 mmol NaCl.

Seedling dry weight: Seedling dry weight was influenced ($p<0.01$) by either salinity levels or cultivars (Table 1). The interactions between cultivars and salinity levels also affected ($p<0.01$) seedling dry weight (Table 1). However, the alteration process of this trait indicated that seedling dry weight of cultivars was reduced by increasing salinity level except Tichung-65. Figure 1 demonstrates the seedling dry weight means of four cultivars over three salinity levels. The highest reduction of Seedling dry weight was observed in Tarom Chaloosi showing 67.54%

Table 1: Variance analysis of traits evaluated in seedling stage over four cultivars at three salinity levels

Source of variation	df	Seedling dry weight	Shoot length	Root length	Seedling wet weight
SL	2	832.126**	428.644	32.436**	149467.146**
Error sl	9	13.852	0.975	1.186	154.049
C	3	175.196**	100.408**	5.186**	27345.632**
SL×C	6	200.288**	17.783**	5.860**	21883.257**
Error c	27	19.702	1.585	0.50	293.771
CV (%)	-	15.86	10.60	12.88	10.26

**: Significant at 1%. SL: Salinity Levels (mmol NaCl), C: Cultivars

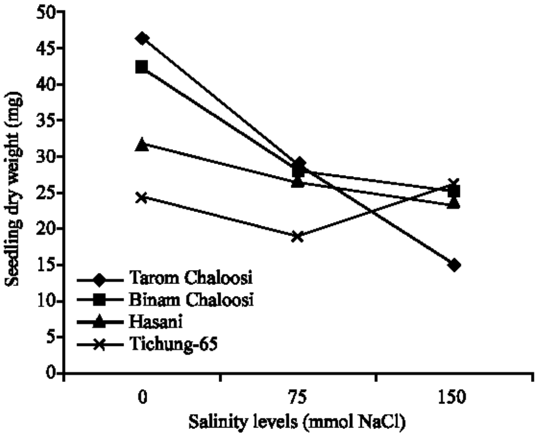

Fig. 1: Seedling dry weight means of four rice cultivars at three salinity levels

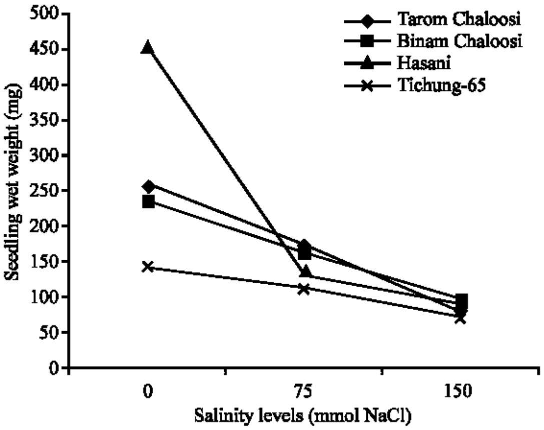

Fig. 2: Seedling wet weight means of four rice cultivars at three salinity levels

reduction at 150 mmol NaCl compared with 0 mmol NaCl whereas, Tichung-65 showed 6.23% increase in this trait as the salinity level increased from 0 to 150 mmol NaCl indicating its ability to grow in saline situation. The reduction rates in Hasani and Binam Chaloosi were 27.21 and 40.82%, respectively. Although Tarom Chaloosi showed the highest seedling dry weight and Tichung-65 the lowest at 0 mmol NaCl, the situation significantly reversed at 150 mmol NaCl. Thus, in 150 mmol NaCl Tarom Chaloosi showed the highest reduction, whereas seedling dry weight of Tichung-65 surpassed the situation no salt concentration added implying the salt tolerance ability and adaptation in salinity stress situation. Schatchman *et al.* (1991, 1992) reported the reduction of seedling dry weight due to the salinity stress so that this reduction has been considered the main standard of evaluating salt tolerance

Seedling wet weight: Seedling wet weight was influenced ($p<0.01$) by both salinity levels and cultivars (Table 1). The interactions between cultivars and salinity levels also affected ($p<0.01$) seedling wet weight (Table 1). Figure 2 demonstrates the seedling wet weight means of four cultivars at three salinity levels. This trait reduced in all cultivars as a result of an increase in salinity level from 0 to 150 mmol NaCl but the reduction process varied among cultivars. Regarding the lowest seedling wet weight of Tichung-65 in both 0 and 150 mmol NaCl, it showed the lowest reduction (50.62%) at 150 mmol compared with 0 mmol NaCl for seedling wet weight whereas Hasani showed the highest reduction (80.2%) at 150 mmol NaCl. Tarom Chaloosi and Binam Chaloosi showed 68.9 and 59.90% reduction, respectively. Although the highest seedling wet weight at 0 mmol NaCl was observed in Hasani, it severely decreased (70.67%) when the salinity level increased to 75 mmol NaCl. But it slightly reduced as the salinity level increased to 150 mmol. It indicates that by increasing salt concentration more than 75 mmol Hasani can relatively adapt itself to the stress although it was significantly influenced by 75 mmol NaCl. Plants initially adjust to saline conditions by decreasing tissue water content through osmotic adjustment (Marschner, 1995). Salinity stress not only influences the plant growth rate through reducing photosynthesis but also considerably reduces plant wet weight by making plant close its stoma accordingly no water penetrates the plant in this way (Redy and Iyengar, 1999).

Shoot length: Shoot length was influenced ($p<0.01$) by both salinity levels and Cultivars (Table 1). The interactions between cultivars and salinity levels also affected ($p<0.01$) shoot length (Table 1). Figure 3 demonstrates the shoot length means of four cultivars at three salinity levels. The results indicated that shoot

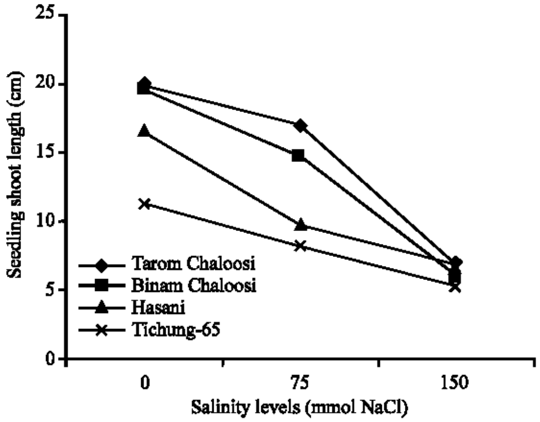

Fig. 3: Seedling shoot length means of four rice cultivars at three salinity levels

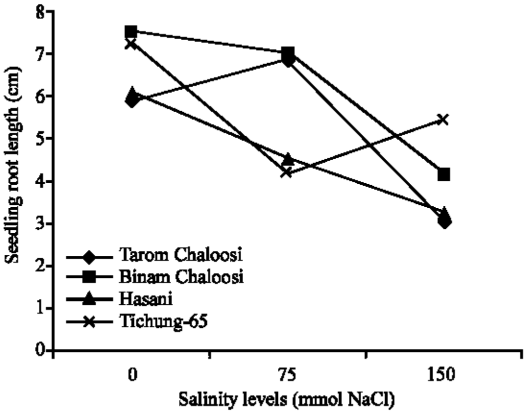

Fig. 4: Seedling root length means of four rice cultivars at three salinity levels

length reduced in all cultivars by increasing salinity level from 0 to 150 mmol NaCl although Tichung-65 and Hasani showed less reduction (53.36 and 58.8%, respectively) compared with 2 other cultivars. Although Tichung-65 showed the lowest shoot length either in 0 or in 150 mmol NaCl, it showed the lowest reduction rate (53.36%) as a result of an increase in salinity level from 0 to 150 mmol NaCl. Therefore, it exhibited the highest tolerance against salinity increase although it was influenced by salinity stress. On the other hand, Binam Chaloosi and Tarom Chaloosi exhibited a sharp reduction (69.12 and 65.87%, respectively) particularly from 75 to 150 mmol. This result indicates their low tolerant capability to increasing salinity stress although they showed the highest shoot length in all salinity levels.

Root length: Root length was influenced ($p<0.01$) by both salinity levels and cultivars (Table 1). The interactions between cultivars and salinity levels also affected ($p<0.01$) root length (Table 1). Figure 4 demonstrates the root length means of four cultivars at three salinity levels. Root length reduced in all cultivars by increasing salinity level from 0 to 150 mmol NaCl. Tichung-65 showed the lowest reduction for root length (29.40%) at 150 mmol NaCl compared with 0 mmol NaCl whereas Tarom Chaloosi, Hasani and Binam Chaloosi showed 47.87, 46.98 and 44.69% reduction, respectively by increasing salinity level from 0 to 150 mmol NaCl. Although root length from 0 to 75 mmol NaCl slightly increased in Tarom Chaloosi, it considerably decreased by more salinity increase from 75 to 150 mmol NaCl. In Tichung-65, on the contrary, root length significantly reduced at 75 mmol compared with 0 mmol NaCl however, by increasing salinity level to 150 mmol root length increased. The highest root length at 150 mmol NaCl was observed in Tichung-65 and the lowest in Tarom Chaloosi, statistically equal to Hasani. These results imply that Tichung-65 is more capable to produce high root length among cultivars as the salinity level increases although it is slightly sensitive in lower salinity stress. Munns (2002) reported that as the salinity stress reduces the growth of seedling and germination rate, evaluating the effects of salinity stress on germination rate and also on seedling root and shoot length is a valid method to assess the salinity tolerance in most of the crops' species. During vegetative growth, plant height, straw weight, root dry weight and root length are all adversely affected by salinity (Gain *et al*., 2004).

Callus volume: This trait was determined through the tissue culture evaluation. The results indicating the effect of different salinity levels on callus volume over cultivars are tabulated in Table 2. This trait was influenced ($p<0.01$) by either salinity levels or cultivars. The interactions between salinity levels and cultivars were also significant ($p<0.01$). Figure 5 demonstrates the callus volume means of four cultivars at four salinity levels. The process of callus volume changes varied among cultivars over the salinity levels but, all cultivars showed a considerable callus volume reduction as they were transferred from 0 mmol to 75 mmol NaCl salinity level. The lowest callus volume of all cultivars was observed in this salinity level. By increasing salinity stress cultivar were relatively adapted to the salinity stress. In this regard, Binam Chaloosi and Tichung-65 were more tolerant than tow other ones and were more capable to grow as the salinity stress increased. Although Tichung-65, like Binam Chaloosi, showed a callus volume reduction in 150 mmol NaCl compared with 0 mmol NaCl, its callus volume increased as the salinity level exceeded 75 mmol NaCl.

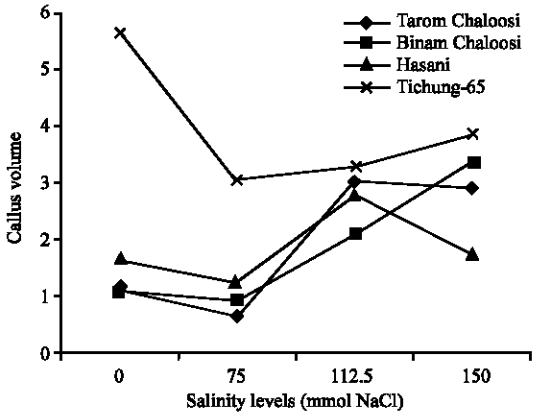

Fig. 5: Callus volume means of four rice cultivars at four salinity levels based on Hooker and Nabors scale

Table 2: Variance analysis of callus volume evaluated at tissue culture stage at four salinity levels over four cultivars

Source of variation	df	Callus volume
SL	3	238.1540**
C	3	552.9148**
SL×C	9	120.8170**
Error	80	0.047
CV (%)	-	8.99

**: Significant at 1%. SL: Salinity Levels (mmol NaCl), C: Cultivars

Sabhashini and Reddy (1998) reported that callus growth rate of rice cultivars was reduced 50% when they were grown in LS medium containing 1 and 2% NaCl (25 and 50% sea water) and was reduced 95% in 3% NaCl (75% sea water). Zair *et al.* (2003) reported that plant regeneration from callus initiated on high NaCl levels may be valid method of selection for salt tolerance.

CONCLUSION

These results indicated that almost all cultivars exhibited a reduction in traits evaluated in greenhouse stage as a result of an increase in salinity levels. However, their susceptibility to the salinity stress was different. Tichung-65 exhibited the lowest reduction and was considered the most salt-tolerant cultivar and Tarom Chaloosi on the other hand, exhibited the highest reduction in most traits as a salt-sensitive one. Besides, only Tichung-65 exhibited an increase in dry weight in 150 mmol compared with 0 mmol NaCl. Its seedling shoot length also increased from 75 to 150 mmol NaCl implying its salt tolerance characteristic and the ability to grow in saline situation. Following it, Hasani was distinguished more salt-tolerant than Binam chaloosi. Thus, cultivar's rank based on their tolerance to salinity stress was Tichung-65 > Hasani > Binam chaloosi > Tarom chaloosi.

The tissue culture experiment also revealed that cultivars such as Tarom chaloosi and Binam chaloosi which were more salt-sensitive in seedling stage, could produce more callus volume and tolerant calli by increasing the salinity level. In addition, cultivars exhibiting tolerance ability to the salinity stress in seedling stage produced more tolerant calli through tissue culture. Tichung-65 had higher salt tolerance characteristic than others. Therefore, since some salt tolerance mechanisms in rice happen in cellular phase, tissue culture technique could be applied for selecting salt-tolerant genotypes. In this regard, more local cultivars showing tolerance ability and more traits should be evaluated. Besides, more comprehensive studies comprising either the whole growing cycle or molecular phase should be carried out in order to select and develop salt-tolerant cultivars.

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