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Response of Transgenic Rice at Germination and Early Seedling Growth Under Salt Stress

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Abstract: The response of germination and early seedling growth of different transgenic rice lines (T-99, T-112, T-115 and T-121) were examined in different levels of salinity (0, 50, 100 and 150 mM NaCl). Final germination, germination rate ($1/t_{50}$, where t_{50} is the time to 50% of germination) and early seedling growth were assessed. Final germination percentage was inhibited with increasing salt concentrations. The required time for germination also increased with increasing salinity levels. The seedling growth was also reduced by salt concentrations, particularly at 150 mM. Root and shoot lengths, root/shoot ratio, fresh weights of root and shoot were also decreased with increasing salt stress. T-99 and T-112 had shown greater performance at germination and early seedling growth as compared to other transgenic lines.

Key words: Germination, seedling growth, transgenic rice, salinity

INTRODUCTION

Rice (*Oryza sativa* L.) is an extremely important staple food for more than one third of world's population. It is estimated that to feed the growing world population total food production will have to increase by 60% in the next 25 years (Khush, 1997). Increasing rice yields has been a goal of agricultural scientists for several decades and many techniques have been developed to achieve this. To improve salt tolerance in rice, recent research has focused on the use of genetic engineering to modify rice plant. Genetic engineering involving the transfer of a gene with a trait of interest can generate transgenic plants with modified traits.

Limited research has been done on salinity tolerance in transgenic rice. Screening procedure for salt tolerance in rice has their own limitations (Aslam *et al*., 1993). Lee *et al*. (2003) reported that the tolerance level of indica rice was higher than that of japonica. Akbar and Yabuno (1974) and Heenan *et al*. (1988) found some japonica varieties with low levels of tolerance during germination, but with improved tolerance at later stages of growth. In recent studies, many diverse japonica varieties have been tested for tolerance during the seedling stage and some tolerant and moderately the seedling stage and some tolerant and moderately tolerant genotypes were identified (Lee, 1995; Lee and Senadhira, 1996; Lee *et al*., 2003). The susceptibility of rice to salinity stress varies with growth stage. Rice is relatively salt-tolerant at germination and in some cases is not affected significantly by up to 16.3 dS m^{-1} of salinity (Khan *et al*., 1997). Rice becomes very sensitive at the young seedling stage, which impacts the stand density in salt-affected fields (Lutts *et al*., 1995).

The hypothesis tested was whether transgenic lines different in ability to sustain seed viability and show normal germination when presoaked in saline solution under laboratory condition with increasing concentration of NaCl.

MATERIALS AND METHODS

Seed treatment: Seeds of different transgenic lines (T-99, T-112, T-115 and T-121) were obtained through crossing between Shanghaixiangrenou and transgenic Line. We used F13 generation's seeds in this experiment. This study was conducted at Sunchon National University, Korea in 2006. Seeds were surface sterilized in 5% NaOCl solution for 10 min, then rinsed with sterilized distilled water. Seeds were blotted dry to remove surface water. Seeds were then soaked for 72 h either in (i) deionized water (control), (ii) 50, (iii) and 100 (iv) 150 mM NaCl solutions. Afterwards, seeds were transferred to two sheets of sterile filter paper moistened with distilled water in sterile Petri dishes and allowed to germinate in the dark in growth chamber at 25±2°C. Petri dishes were sealed with parafilm to prevent evaporation of water, thus minimizing the changes in concentration of the solutions. The temperature of the growth chamber was maintained at 25±2°C. Seedlings were harvested and shoots and roots of seedlings were separated on day 9.

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Growth parameters: Seed germination and germination rate ($1/t_{50}$, where t_{50} is the time to 50% of germination) were evaluated after every 12 h upto day 9. Seeds were considered to be germinated with the emergence of the radicle. A total of 20 seedlings from each treatment were sampled randomly at day 7 and shoots and roots were separated. Lengths were measured and Fresh Weights (FWs) of shoots and roots were recorded.

The experiment was designed by using a randomized complete block design with five replications. Analysis of variance was performed by using the Microsoft Excel. Means values for different plant growth parameters were compared through LSD test.

RESULTS

The germination response of transgenic lines was compared under various salt stress condition (Fig. 1A). When the salt concentration exceeded, the germination of the transgenic lines were significantly decreased. The transgenic lines with the highest relative germination at 150 mM NaCl were T-99 and T-112. The lowest germination was observed in T-121 at high salt concentration. Germination rate of seeds of different transgenic lines under various conditions of salt stress was expressed as a $1/t_{50}$ of the germination of seeds of the same population as in control. The germination response of the different transgenic lines under investigation showed considerable differences in the initiation and completion of germination. Germination started within 48 hours and was complete on the 9th day. Figure 1B indicated that T-99 and T-112 completed their germination at approximately the same time, but T-121 took comparatively more time to complete germination especially at the high salt treatments (Fig. 1B).

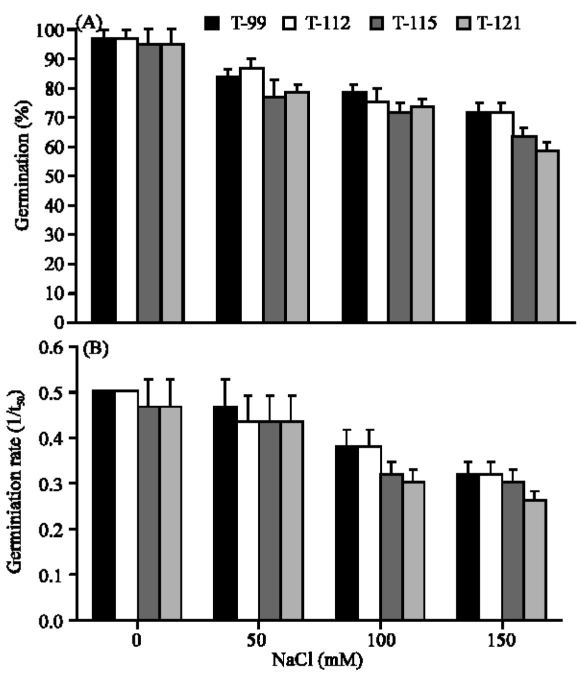

Fig. 1: Effect of NaCl on germination (A) and germination rate (B) in different transgenic rice lines

Further observations were carried out to observe the effect of NaCl on early seedling growth of different transgenic lines. The growth of all transgenic lines was inhibited with increasing NaCl concentration (Fig. 2 and 3). The results showed that the increase in NaCl concentration delayed emergence of root and shoot as compared to control. The result presented in Fig. 2 showed a significant variation in plant root and shoot length and root/shoot ratio. With increasing NaCl concentrations, the root and shoot lengths of transgenic rice seedlings decreased (Fig. 2). Root length reduction was more pronounced in T-121 while least reduction was observed in T-112 (Fig. 2A). T-112 also showed less

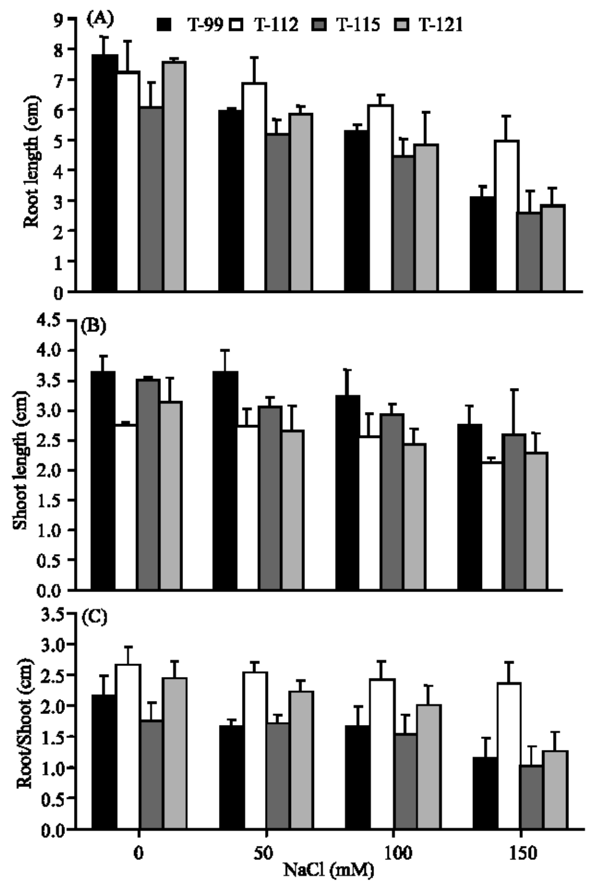

Fig. 2: Effect on NaCl on root length (A), shoot length (B) and root/shoot ratio in different transgenic rice lines

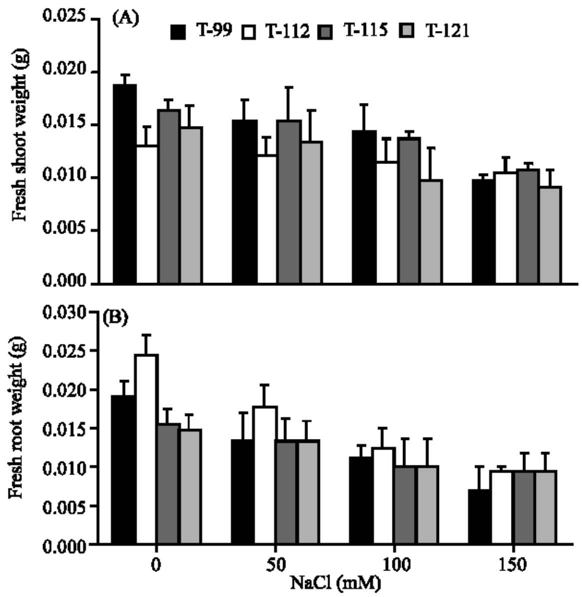

Fig. 3: Effect on NaCl on fresh weights of root (A) and shoot (B) in different transgenic rice lines

reduction in shoot length. Maximum reduction in shoot length was measured in T-121 (Fig. 2B). Higher root/shoot ratio was observed in T-112 while lowest root/shoot ratio was investigated in T-121 (Fig. 2C).

With the increasing concentration of NaCl, significant reduction was observed in mean fresh weights of root and shoots of transgenic lines (Fig. 3). However, the transgenic lines differed in these growth parameters in response to increasing salt concentration. T-112 showed greater response with respect to fresh shoot weight. The decrease in fresh weight of shoot was more pronounced in T-121 (Fig. 3A). The transgenic lines with the highest fresh weight of root were T-112, T-115 and T-121 while lowest fresh root weight was observed in T-99 under high salt concentration (Fig. 3B).

DISCUSSION

Tolerance at emergence is based on survival, whereas tolerance after emergence is based on decrease in growth or yield (Maas, 1986). In the context of this discussion, the term salt tolerance during seed germination is measured on germination percentage and germination rate under salt stress conditions. Salt tolerance during seedling growth is assessed on the basis of root and shoots length and fresh weights of root and shoot at a given salt concentration. On the basis of these two criteria, our results showed variation in seed germination and early seedling growth responses to NaCl in different transgenic lines. Present results indicated that T-99 and T-112 had superior germination performance under high stress condition. These lines were also being able to germinate rapidly both under control and salt stress conditions (Fig. 1). The suppression of germination at high salt levels might be mainly due to osmotic stress (Heenan *et al*., 1988). Folkard and Wopereis (2001) reported that salinity delayed germination in rice with increasing salt stress.

Seedling survival appears to be a useful parameter to characterize individual and varietal differences owing to its objectivity and ease of measurement (Flower and Yeo, 1981). Later vegetative growth is of much less use as an indicator of salt resistance since it is very much less sensitive to salinity than is grain yield (Akbar and Yabuno, 1974). T-112 had higher root and shoot length than the other transgenic lines. The transgenic lines such as T-121 was the lowest in root and shoots length at high salt concentration (Fig. 2). T-112 showed greater response with respect to fresh shoot and root weight as compared to other the transgenic lines (Fig. 3). It was also observed that seedling growth reduced with increasing salt stress. Present results are similar with Heenan ***et al.*** (1988) and Lutts ***et al.*** (1995). They observed that young seedling were very sensitive to salinity in commonly cultivated rice. Lee ***et al.*** (2003) also reported that percent root reduction was the most affected by salinity stress in all varieties followed by shoot dry weight then by seedling height.

It was concluded that germination and early seedling growth of different transgenic rice lines were also inhibited by increasing salt concentration. Although T-99 and T-112 showed good response under high salt concentration as compared to other lines.

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