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Full Length Research Paper

The effect of NaCl stress on the germination of seed and growth of wild species and cultivated varieties of tomato

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In this study, a cultivated species (*Lycopersicon esculentum* 'Moneymaker') and a salt-tolerant wild species of tomato (*Lycopersicon pimpinellifolium* 'PI365967') were used to study growth characteristics within solutions of varying NaCl concentrations. Results show that the germination rates of PI365967 and Moneymaker were 100% under concentrations of 0 mmol/L NaCl. The germination of both types of plants delayed when treated with a 100 mmol/L NaCl conditional, but the germination rate of PI365967 was always significantly higher than Moneymaker. In the seedling growth period, the growth of roots and aerial parts of PI365967 and Moneymaker were inhibited under NaCl stress. However, the latter suffered a greater extent of inhibition than the former. Under a 200 mmol/L NaCl treatment, the growth of Moneymaker was strongly inhibited, and accompanied by chlorosis, but PI365967 maintained a good growth trend. Moreover, the degree of inhibition was small. Whilst gradually increasing the concentration of NaCl to 200 mmol/L, we found that the adaptation of the two types of tomato to salt stress increased (35 days after treatment). The plants of Moneymaker treated directly with concentrations of 200 mmol/L NaCl all died, but the plants treated with a gradual increase in NaCl concentration continued to grow at a slow rate. PI365967 had new lateral roots and buds grew as well. The results indicate that appropriate exercise of the salt stress can improve the salt-tolerance of tomato. It should be noted that PI365967 had greater capability of salt-tolerance.

Key words: Wild species and cultivated variety of tomato, germination of seed, growth of seedling, NaCl stress, salt-tolerant.

INTRODUCTION

Salt stress is one of the major environmental stress factors that affect plant growth and development (Lu, 2010). About 20% of the world's land suffers from salinization, and nearly 50% of the irrigated land is influenced by secondary salinization (Tanj, 1990). China has 20000000 hm^2 of salinized land, which accounts for about 25% of the arable land area. At the same time, inappropriate agricultural methods also cause a lot of secondary salinization (Tester and Davenport., 2003; Li, 2007). Salinization has become one of the most important environmental factors that affect agricultural development (Shono et al., 2001). Therefore, finding methods to lessen secondary salinization and improving salt-tolerance of crops have become widespread concerns (Tanj, 1990; Tester and Davenport., 2003).

Saline-alkali soil can be improved by planting salt-tolerant plants (Wu et al., 2008). Cultivating such plants to carry out development and utilization is amongst effective measures to recover salinized land (Liu and Wang, 2011). The tomato (*Lycopersicon esculentum* Mill),

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Abbreviations: NPN, Non-protein-nitrogen; **TFAA,** total of free amino acids.

belonging to the family Solanaceae and the genus Lycopersicon, is an annual herb that adapts poorly to salinized soil. Secondary salinization of soil severely affects the quantity and quality of vegetables, and has become one of the main problems encountered in the tomato cultivation (Ma, 2008). Extensive research on salt-tolerance species of tomatoes has been conducted both at home and abroad (Gong et al., 1990). The identify-cation of salt-tolerant species is fundamental for further breeding (Meng et al., 2007). Gong Ming pointed out that salt-tolerance is lowest in the stages of germination and seedling, while plants are relatively insensitive to salt stress within other stages (Gong et al., 1994). Liu et al. (2006) suggested that the relative growth of plants may reflect the strength/weakness of the overall salt-tolerance.

In this study, the effects of NaCl stress on seed germination and seedling growth of a salt-tolerant wild species and a cultivated variety of tomato were studied in order to reveal the mechanism of salt stress, and to provide a theoretical basis for breeding, identifying, screening and cultivating salt-tolerant varieties of tomato.

MATERIALS AND METHODS

The cultivated tomato (L. *esculentum* Moneymaker) and salt-tolerant wild tomato (L. *pimpinellifolium* PI365967) were used as plant material for this experiment. Seeds were provided by the laboratory of Dr. Li Junming, Institute of Vegetables and Flowers, Chinese Academy of Agricultural Science.

Seed germination rate

Seeds were washed for 2 min with 70% ethanol and treated in a 50% solution of sodium hypochlorite (cultivated tomato was treated for 10 min and wild tomato for 30 min), then washed five times with sterile water. Under asepsis conditions, seed were placed in Petri dishes on three-layered filter paper moistened with 10 ml distilled water for germination. NaCl concentration gradient was set to 0 and 100 mmol/L. Germination was recorded in detail. Each processing set was repeated three times; each repeat sowing 20 seeds.

Seedling cultivation

The seedlings of tomato were planted in vermiculite after 7 days of germination and placed into 1/4 Hoagland solution in 3 to 4 days for recovering; then 0 mmol/L and 200 mmol/L NaCl solution were used to treat the tomato seedlings.

Parameters measured

The germination rate was measured according to the germination percentage of 7 days after sowing seeds; plant height and root length parameters according to the experimental setting time, with conventional measuring methods. Standard for seed germination was 0.2 cm radicle length. The seed germination statistics from the 2nd day to the end of the 7th day was recorded using the formula:

$$\text{Germination rate (GR) (\%)} = \frac{\text{Number of seeds germinated within the specified date}}{\text{Number of seeds tested}} \times 100\%$$

RESULTS

Effect of NaCl stress on the germination percentage of wild species and cultivated variety of tomato

Under 0 mmol/L NaCl conditions, the germination time of PI365967 seeds were shorter than those of Moneymaker. The germination of all PI365967 seeds occurred on the fourth day, while that of Moneymaker was on the fifth day. Under 100 mmol/L NaCl conditions, the time of PI365967 and Moneymaker seed germination were both delayed. Figure 1 shows that on the fourth day, the germination rate of PI365967 was 92.5%, while that of Moneymaker was only 70%. And on the fifth day, the germination rate of PI365967 reached 100%, but that of Moneymaker was only 85% at this time.

The effects of NaCl stress on the morphology wild species and cultivated variety of tomato

The Hoagland's mediums containing 0 and 200 mmol/L of NaCl were used to treat tomato seedlings in the 6-leaf stage. In 200 mmol/L NaCl conditions, the two types of tomato seedlings were within 30 min lodging and resumed standing in the 48 h, and it was found that recovery of PI365967 was much faster than Money-maker. The growth of Moneymaker treated with 200 mmol/L NaCl was strongly inhibited and accompanied by yellowing, but PI365967 still maintained a good growth trend. Moreover, the leaves stayed relatively green; the inhibition degree was also less than Moneymaker (Figure 2).

Influence of NaCl stress on the seedlings height of wild species and cultivated variety of tomato

The plant heights of two species tomato were statistically significant (Figure 3). The results show that with the time extension of salt stress, the height of PI365967 did not change much in solutions of 200 mmol/L of NaCl ($P>0.05$), but the height of Moneymaker was reduced significantly ($P<0.01$) and it slowly withered. On the twenty-ninth day, Moneymaker died completely, while only one tomato seedling of PI365967 withered.

The effect of NaCl stress on seedling root length of wild species and cultivated variety of tomato

At the same time, the root lengths of the two species were also measured. Figure 4 shows that the root length of two species tomato seedling treated with 0 mmol/L NaCl elongated on the tenth day. However, the roots of the seedlings treated with 200 mmol/L NaCl did not elongate. Overall, the growth of both roots were

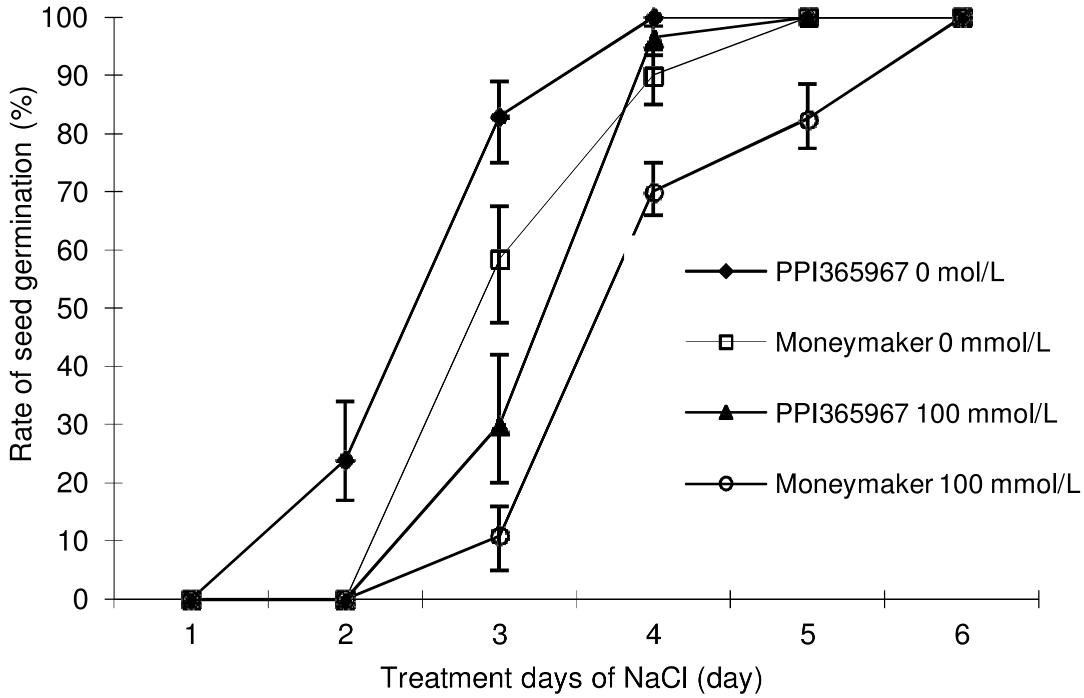

Figure 1. Characterization of seed germination of PI365967 and Moneymaker, treated with 100 mmol/L NaCl solution and 0 mmol/L NaCl condition.

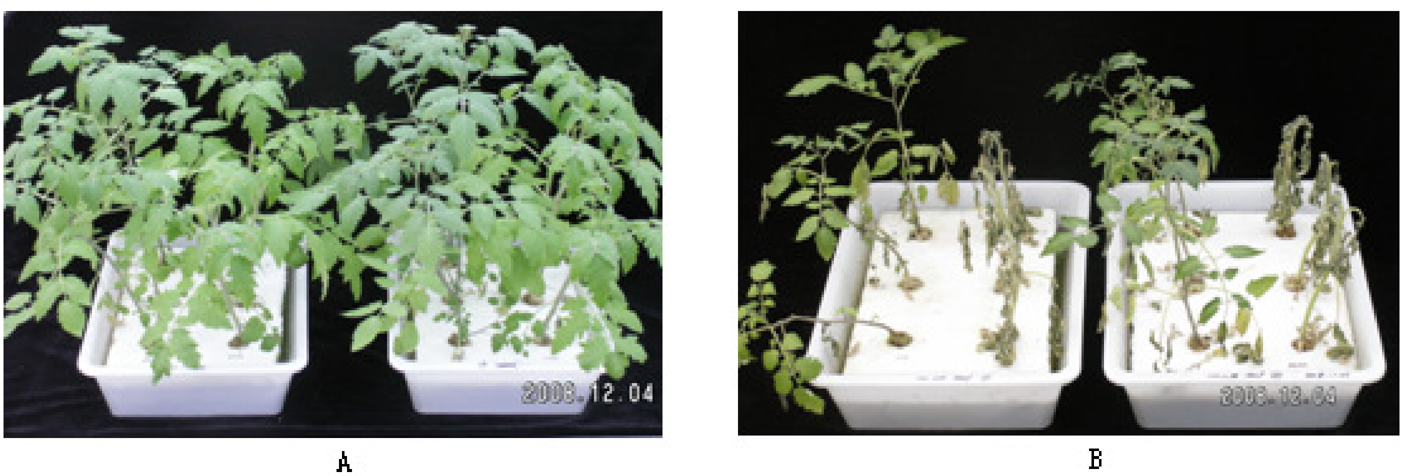

Figure 2. The relative decrease of shoot growth of salt-tolerant tomato genotype PI365967 and a moderate-tolerant genotype Moneymaker, treated respectively with 200 mmol/L NaCl solution (A) and in normal condition (B) for 10 days. Left rank in the hydroponic tank is PI365967, while the right one is the Moneymaker variety.

significantly inhibited ($P<0.05$). By the twenty-first day; the root length of Moneymaker and PI365967 were shorter than original. Comparatively, the shortening degree of Moneymaker (30.7%) was relatively faster than that of PI365967 (15.3%). The relative elongation rate of root was given as: Elongation rate of root = Root length of twenty-first – the root length of 0 days / the root length of 0 days.

It is worth mentioning that starting from the thirteenth day, the plants of PI365967 treated with 200 mmol/L NaCl gave rise to new buds at the bottom of the plant (16^{th} day), had fresh lateral white roots growing, and on the 35^{th} day, length and number of lateral root were increased. The buds also grew into leaves. However, in this process, and until death, Moneymaker sprouted no new leaves or roots. Therefore, we can conclude that

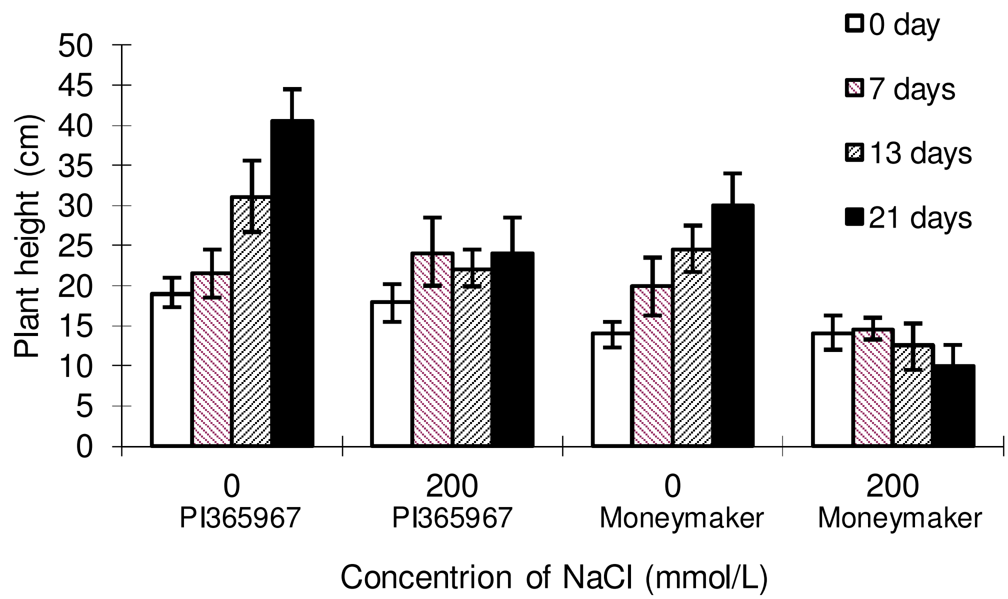

Figure 3. The plant height of PI365967 and Moneymaker treated respectively with 0 and 200 mmol/L NaCl solution.

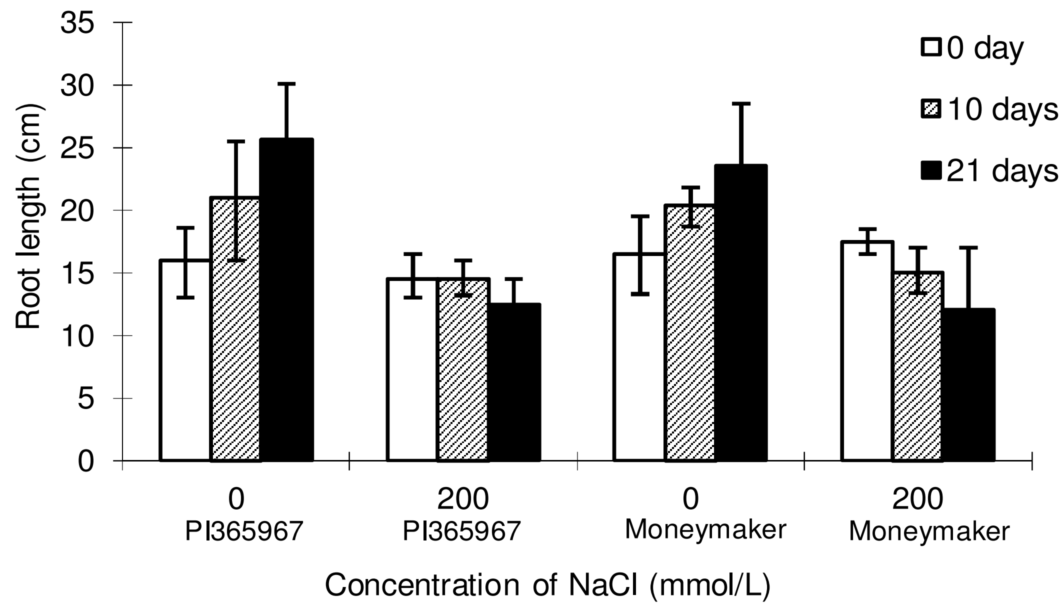

Figure 4. The root length of PI365967 and Moneymaker treated respectively with 0 and 200 mmol/L NaCl solution.

under 200 mmol/L NaCl stress, PI365967 has higher salt-tolerance and adaptability compared with Moneymaker.

The effect of stress increased NaCl by steps on seedling growth of wild species and cultivated variety of tomato

Out of curiosity about how PI365967could adapt to salt stress better than Moneymaker, we also made comparative research by (a), using 200 mmol/L NaCl directly and (b), gradually increasing NaCl to 200 mmol/L NaCl for stress treatment (salt 25 mmol/L added daily). It was found that the performances of the two species of tomato under these different conditions differed spontaneously (Figure 5). From Figure 5, it can be clearly seen that the Moneymaker plants with immediate use of 200 mmol/L NaCl treatment (Figure 5; bright column plants in the

Figure 5. The relative decrease of shoot growth of salt-tolerant tomato genotype PI365967 and a moderate-tolerant genotype moneymaker treated respectively with NaCl solution gradually (A) and 200 mmol/L NaCl solution (B) for 16 days. The left rank in the hydroponic tank is PI365967, while the right one is a Moneymaker variety.

sink) have all wilted, and were close to dying; while the Moneymaker plants of an increase by steps until 200 mmol/L NaCl treatment (Figure 5A; right column plants in the sink) only grew at a relatively slow rate and the growth trend was still maintained. In both conditions, the whole growth performance of PI365967 (Figure 5A and B left column plants in the sink) exceeded that of Moneymaker.

Similarly, the height and root length of PI365967 and Moneymaker were measured when increasing the concentration of NaCl step by step to 200 mmol/L. We found that even when gradually increasing NaCl to 200 mmol/L, there is still some inhibition for the growth of PI365967 and moneymaker, but the inhibition intensity decreased ($P > 0.05$) (Figure 6). Notably, the height of Moneymaker did not decrease but increased slowly instead, and kept a stable level (Figure 6). On the 35th day, Moneymaker survived, whereas the Moneymaker dealt with 200 mmol/L NaCl directly all died. Furthermore, the root growth inhibition of PI365967 and moneymaker also reduced when gradually increasing the concentration of NaCl to 200 mmol/L. Compared with the control, the difference of root lengths was not significant ($P>0.05$) (Figure 7), but PI365967 still showed stronger salt-tolerance than Moneymaker. The results show that PI365967 still maintained a good growth trend. New lateral roots and buds were continuously growing while increasing NaCl concentration gradually to 200 mmol/L indicates that PI365967 had a strong ability to adapt to salinization. However, in this case, growth of Moneymaker was slow and finally almost stagnant. In summary, PI365967 showed higher tolerance and adaptation to salt stress in both cases.

DISCUSSION

Salt damage is one of the most important abiotic stresses in agricultural production during the stage of seed germination (Ma and Cui, 2006). The circumstances of seed germination under salt stress can reflect a plant's salt resistance to a certain extent (Van der, 1980). Asish (2004) concluded that in different concentrations of salt stress, the circumstances of seed germination and salt-tolerance of plants themselves have some relations during germination. Whether plants adapt to saline-alkali habitat primarily depends on the germination rate and vigor of seedlings after germination (Zhang et al., 2009). The seed germination rate, relative germination rate, germination potential and germination index are important indicators to evaluate the strength or weakness of a plant's salt-tolerance during seed germination (Mu et al., 2009). According to related research, the germination rate, germination potential and vitality index of salt-resistant plants in general are higher than those of salt sensitive plants (Ding et al., 2001; Kumar et al., 1988; Sharma and Yamdagni, 1989).

Seed germination rate is significantly inhibited by salt stress, and the degree of inhibition increases with the increase of salt concentration. The rate of seed germination under salt stress can be recognized as the basis for determining salt-tolerant of plants. In this research, the PI365967 and Moneymaker in seed germination under NaCl stress were first compared. The seed germination time of PI365967 and Moneymaker were both delayed in 200 mmol/L NaCl conditions (Figure 1). However, the seed germination of PI365967 was faster than that of Moneymaker, being one day faster during

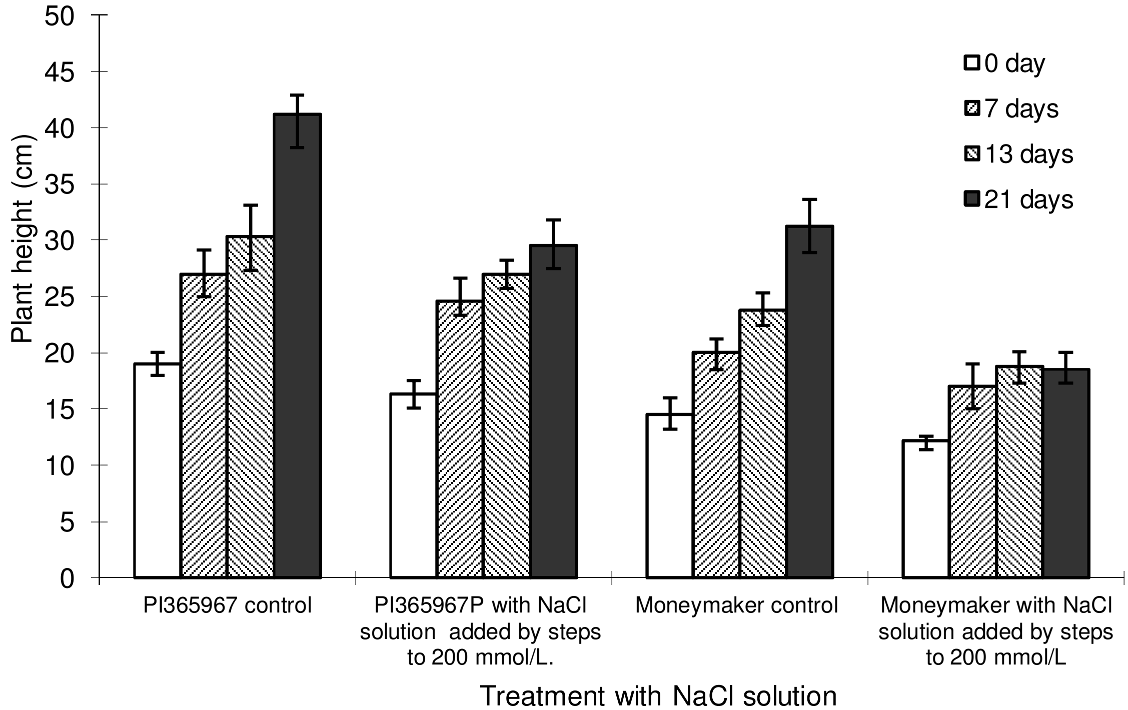

Figure 6. The plant height of PI365967 and Moneymaker treated respectively with 0 mmol/L NaCl solution added by steps to 200 mmol/L.

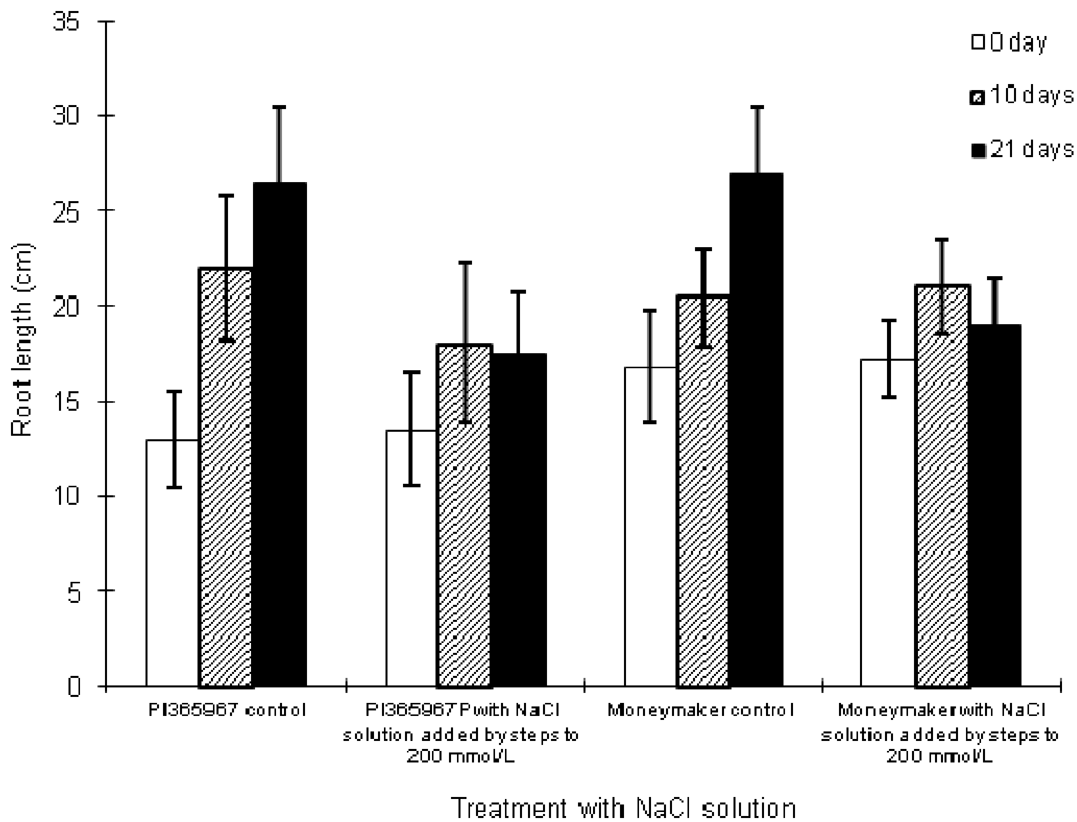

Figure 7. The root length of PI365967 and Moneymaker treated respectively with 0 mmol/L NaCl added by steps up to 200 mmol/L.

germination. Under the same salt concentration stress, the relative germination rate of PI365967 was higher than that of Moneymaker (P <0.01). It was indicated that PI365967 is more salt-tolerant than Moneymaker. Growth is the most sensitive physiological process of plants to saline response (Yan et al., 2009). The normal growth of plants is significantly inhibited by salt stress (Zhan et al., 2006). If the plant is not adapted to adversity, growth will be reduced and it will die on serious occasions, while plants of tolerance only exhibit a decrease of growth speed. The plant growth and survival rate are a basis for judgment of the tolerance ability to saline alkali stress because they can represent integrated performance of many physiological mechanisms of plants (Niknam, 2000).

Under the direct use of 200 mmol/L NaCl stress, the growth of PI365967 showed greater tolerance and adaptability than Moneymaker. The growth of Moneymaker was severely inhibited; it slowly turned yellow, withered and eventually died. However, the growth of PI365967, although inhibited, the degree of inhibition was much smaller than that Moneymaker nonetheless; and in this period, PI365967 had new roots and grew leaves, indicating that PI365967 has certain adaptability to salt stress. With the gradual increase of NaCl stress to 200 mmol/L, it was found that Moneymaker also showed a certain degree of salt adaptation. The growth was only somewhat inhibited (unlike the results of instant use of 200 mmol/L NaCl stress), indicating that appropriate exercise of salt stress can improve salt-tolerance of tomato. We also found that when increasing the concentration of NaCl by steps to 200 mmol/L, PI365967 showed better growth than when directly using 200 mmol/L NaCl. Thus, PI365967 showed a more significant salt tolerance

There is a vast number of wild tomato species that belong to Lycopersicon, which is close genetically to the cultivated tomato. This provides very good material for us to study the mechanism of salt-tolerant of tomato. As far as we know, the salt-tolerance of the wild tomato, *L. pimpinellifolium* (a.k.a PI365967), has not yet been reported. From our experiments, PI365967 is a wild tomato species of high salt-tolerance. We intend to continue our study on PI365967 and Moneymaker to find out regulations of differences that occur within gene expression which in turn provokes response of plants to salt stress. In so doing, we hope to achieve our goal of (a) fully understanding the key metabolic pathways and regulatory mechanisms regarding salt tolerance of the species of wild tomato mentioned above, and (b) eventually applying these technologies to cultivate salt-tolerant species of tomato.

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