Soaking Rice Seeds in High Concentration Salt Solution Improved Salt Tolerance at the Seedling Stage

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Abstract [**Objectives**] This study was conducted to enhance the salt tolerance of current rice varieties at the seedling stage and fulfill the urgent requirement for salt-tolerant rice varieties in coastal tidal flats. [**Methods**] Four high-generation stable rice lines with diverse salt tolerance were employed as test materials, and four NaCl concentration gradients were established for seed soaking treatment. [**Results**] The seedling survival rate of line 151465 underwent significant alterations after soaking with four different salt concentrations, and the survival rate was the highest after treatment with 1.8% NaCl for 1 d, reaching 65.2%. The average survival rate of other three lines with different salt tolerance reached 62% after soaking with 1.8% NaCl for 1 d, which was significantly higher than those of the 2.2% NaCl and 0% NaCl treatments. [**Conclusions**] This study provides a basis for reducing the effect of abiotic stress on rice growth and development and improving the utilization rate of saline-alkali land.

Key words Rice; Salt water; Seed soaking; Salt tolerance; Seedling stag

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Under the situation that existing cultivated land resources in China have been fully excavated and the productivity level of conventional cultivated land is facing bottlenecks, it is of great practical and long-term strategic significance to strengthen the development and utilization of coastal tidal flats to enhance the agricultural production capacity of cultivated land in China and ensure national food security. China's coastal beaches are located in the estuaries of major rivers such as the Yangtze River, the Yellow River, the Huaihe River, the Pearl River and Heilongjiang. Because there are abundant fresh water resources, reclaimed coastal tidal flats are conducive to rice planting. During the development and utilization of coastal tidal flats, due to the special water environment during the growth of rice, tidal flat surface covered by water can play a role in leaching, salt compression and salt reduction of soluble salts and alkalis in soil, and it can also play a role in balancing soil fertility. In addition to the unique biological role of rice in reducing salt, planting rice on saline-alkali land has become an effective way to improve the physical and chemical properties of soil, which can not only promote the rational use of land resources, but also become a powerful hand for farmers to increase income and become rich[1].

In view of the present situation that there are not many salt-tolerant rice varieties in coastal tidal flats of China, and existing salt-tolerant rice varieties have low yield, poor disease resistance, especially rice blast resistance, and poor quality, screening salt-tolerant rice varieties or improving the salt tolerance of existing varieties (lines) through technical means can meet the urgent demand for salt-tolerant rice varieties in coastal tidal flats. Throughout the entire growth cycle of rice, different growth stages show varying levels of salt sensitivity. Rice seedlings have the strongest salt sensitivity[2] and are most susceptible to salt stress. At this stage, rice is most sensitive to salt and has the weakest salt tolerance. In the future, as rice grows, its salt tolerance gradually increases, and the damage will also be reduced[3].

Materials and Methods

Experimental materials

Stable high-generation lines 151465, 151425, 151342 and 151343 with difference salt tolerance selected by our team were used as test materials. All experimental materials were planted in the Lishui Experimental Base of Jiangsu Academy of Agricultural Sciences, following conventional planting methods. After harvesting and drying, the seeds were stored for future use

Experimental methods

Determination of salt treatment time and concentrations

Line 151465 with moderate salt tolerance was selected for the experiment, which was set with four salt concentration gradients, *i.e.*, 0%, 0.6%, 1.2% and 1.8%. The seeds of 151465 were soaked in the four salt concentration gradients for 1 and 2 d, and then washed to remove salt and transferred to fresh water to accelerate germination. When the seedlings grew to three leaves and one heart, they were transferred to nutrient solution containing 0.5% NaCl for growth again, and the survival rate of the seedlings was investigated 21 d later. The experiment was set with two replicates, each of which involved 48 seedlings.

Seed soaking treatment with high-concentration salt water

In order to further determine the effects of high salt concentration

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on the salt tolerance of rice seedlings, three other lines of different salt tolerance types, 151425, 151342 and 151343, were treated with the above-mentioned salt concentrations and treatment time. Then, the salt tolerance of rice seedlings was identified according to the above-mentioned method.

Results and Analysis

Determination of salt treatment time and concentration

The seedling survival rate of line 151465 changed obviously after being treated with four salt concentrations. The average survival rate of seedlings treated with 1.8% NaCl for 1 and 2 d was 60% (Table 1), and the survival rate of seedlings treated with 1.8% NaCl for 1 d was the highest, reaching 65.2% (Fig. 1).

The number of days of treatment also had a significant impact on the seedling survival rate of line 151465, and the seedling survival rate of seed soaking treatment for 1 d was significantly higher than that of treatment for 2 d (Table 2).

Table 1 Effects of different salt concentrations on seedling survival rate of rice line 151465

Salt concentration	Average value//%	5% significant level	1% significant level
1.8% NaCl	60	a	A
1.2% NaCl	45	b	B
0.6% NaCl	40	c	C
0% NaCl	29	d	D

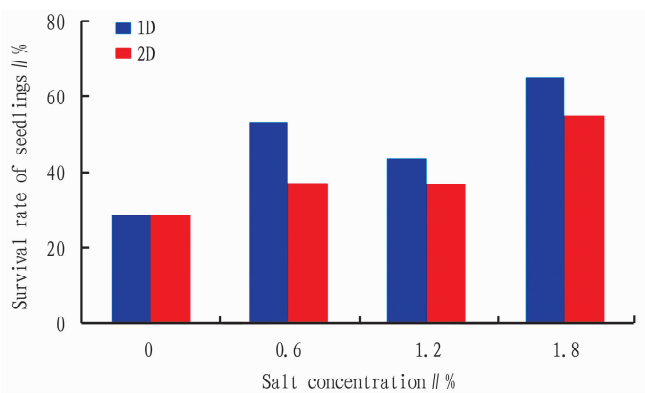

Fig. 1 Survival rate of seedlings under different salt concentrations and treatment time

Table 2 Effects of different treatment time on seedling survival rate of line 151465

Days//d	Average value//%	5% significant level	1% significant level
1	48	a	A
2	39	b	B

Effects of high salt concentration treatments on survival rate

After soaking seeds with 1.8% NaCl for 1 d, the average survival rate of the three lines with different salt tolerance reached 62%, which was significantly higher than those of the 2.2% NaCl and 0% NaCl treatments (Table 3). After soaking seeds with high concentrations of salt for 1 d, the seedling survival rates of all three lines were greatly improved, and the increase of the 1.8% NaCl seed soaking treatment was even greater than that of the 2.2% NaCl seed soaking treatment (Fig. 2).

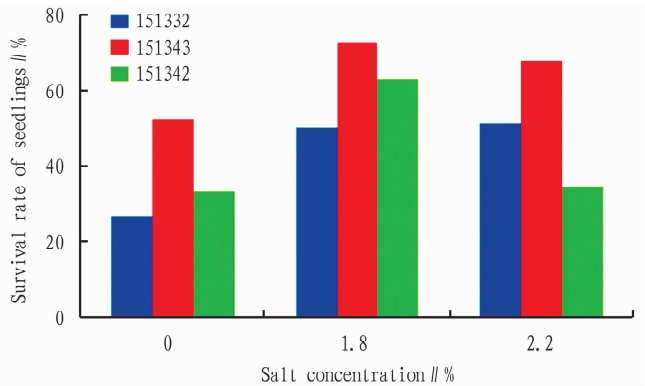

Fig. 2 Survival rate of seedlings under different salt concentrations and treatment time

Table 3 Effects of high salt concentrations on seedling survival rate of three lines

Salt concentration	Average value//%	5% significant level	1% significant level
1.8% NaCl	62	a	A
2.2% NaCl	51	b	B
0% NaCl	37	c	C

Effects of high salt concentrations on germination rate of rice seeds

In order to find out the changes in germination rates of three lines after soaking in high-concentration salt solutions for 1 d, the germination rate of each line was investigated and statistically analyzed. The results showed that there was no significant difference between the 1.8% NaCl and 2.2% NaCl treatments, but the germination rates were significantly higher than that of the 0% NaCl treatment (Table 4), that is, the germination rate increased after soaking seeds with high concentration of salt for 1 d (Fig. 3).

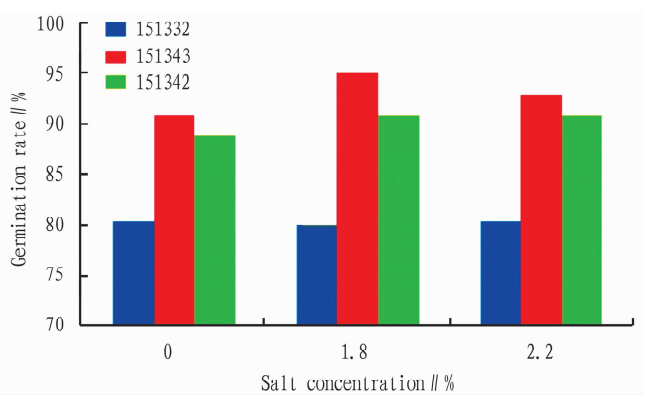

Fig. 3 Germination rate of rice seeds treated with different salt concentrations

Table 4 Effects of high salt treatments on germination rate of three lines

Salt concentration	Average value//%	5% significant level	1% significant level
1.8% NaCl	94	a	A
2.2% NaCl	93	a	A
0% NaCl	92	b	B

Conclusions and Discussion

1.8% NaCl treatment significantly improved the salt tolerance of rice varieties at the seedling stage

According to the above results, after 1.8% NaCl treatment, the seedling survival rates of all tested lines were significantly improved. In specific, the seedling survival rate of line 151465 increased by 128%, and the values of lines 151425, 151343 and 151342 increased by 87%, 40% and 89%, respectively. The 2.2% NaCl salt treatment could also improve the seedling survival rate of rice lines, but the increase rate was smaller than that of the 1.8% NaCl treatment, indicating that the 1.8% NaCl seed soaking treatment was the best salt concentration.

Soaking seeds with high concentrations of salt for 1 d could significantly improve the germination rate of rice seeds and the salt tolerance of rice seedlings

Through the analysis of the effects of different salt treatment time on the salt tolerance at the seedling stage of strain 151465, the average survival rate of seedlings treated for 1 d was 48%, which was significantly higher than 39% of seedlings treated for 2 d (39%), indicating that the appropriate salt soaking time was 1 d.

After soaking the seeds in 1.8% NaCl solution for 1 d, the average germination rate of the three lines was 94%, which was extremely significantly higher than that free of salt water soaking.

Adversity training could improve the adaptability of plants or their ability to cope with adversity

The earliest research on the training effect of plants mainly focused on the adversity training effect induced by biological stress. Pretreatment of plants with sublethal dose of pathogenic bacteria can improve the resistance of plants to pathogenic bacteria[4-9]. As an active strategy to cope with adversity, adversity training has great application potential in production experiments, so it has attracted more and more attention[10]. Based on the type and time of subsequent abiotic stress, pretreating seeds with concentrated salt water in this study belong to the same type of adversity training effect in contemporary times, that is, the adversity training is the same as the subsequent actual stress. It can be seen by analyzing previous studies on contemporary training effects that there are still many aspects to be clarified in the pretreatment of adversity training, such as the stage, degree and duration of adversity pretreatment. In this study, it was also found that there were significant differences among different salt water concentrations, different pretreatment days and different lines. Among them, soaking seeds in 1.8% NaCl solution for 1 d achieved the best effect on the salt tolerance of rice varieties at the seedling stage.

The mechanism of salt stress training of rice needs further study, so as to provide a basis for practical salt stress training with obvious effect, reduce the effect of salinization abiotic stress on rice growth and development, and improve the utilization rate of saline-alkali land.

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(Continued from page 15)

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