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Full Length Research Paper

Response of transgenic rice at germination traits under salt and alkali stress

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Rice is a worldwide cereal crop. Rice yield is severely threatened by saline and alkali stresses. A transgenic rice variety and its wild type (Nipponbare) were treated with four different levels of NaCl / Na_2CO_3 solutions to study the germination traits (germination percentage, root length, dry weight) of transgenic rice. Rice was found to be more sensitive to Na_2CO_3 than to NaCl solutions. Germination percentages declined with increasing sodium salts. When treated with 1.5% NaCl, only 40% of Nipponbare and 51% of transgenic rice could survive. The root length of Nipponbare was significantly more restricted than that of transgenic rice. Transgenic rice showed better performance at germination and early seedling growth compared to its wild type under salt and alkali stress.

Key words: Transgenic rice, saline stress, alkali stress, germination traits.

INTRODUCTION

Rice is the most important crop in Asia and a staple food for over half of the world's population. The demand for increased rice production requires the development of new cultivars with higher yields, and improved tolerance to abiotic stress as global environment getting more complicated and deteriorated. Salt and alkali are two major abiotic stresses leading to the reduction of rice yield (Tuteja, 2007). According to incomplete statistics, 950 million ha of the world's land area are saline-alkali soil (Zhang et al., 2006). Salinity is a very critical factor regulating agricultural productivity worldwide. Accumulation of soluble salts of various types, mainly the chlorides of sodium, calcium, magnesium, and sulphates or carbonates, induces soil with high osmotic potential and ionic toxicity. One of the most poisonous ions is sodium salt. Excessive ions within cells results in dynamic changes of many enzymes, and this leads to an overall decline in germination and seedling growth (Shao et al., 2005). Salinity also causes a series of composition changes, permeability, transportations, and ion flows of plasma membranes. These result in defects in cell metabolism and function. Alkaline soils are formed by the accumulation of $NaHCO_3$ and Na_2CO_3, which once hydrolyzed, would raise the pH of soil to above 9.0. Alkalinity results in a hardened, poorly ventilated soil, a place where not suitable for rice to grow (Sengupta et al., 2010). The transgenic approach provides new opportunities for rice to live through environmental or non-environmental stresses. In the past two decades, tremendous progress has been made in transgenic rice research with respect to the most important traits, including insect and disease-resistance, abiotic tolerance, quality, and yield potential (Chen et al. 2009). In a previous study, we obtained a 796 bp promoter of the PR10 gene from *Pinus strobes* roots (Liu et al. 2003), constructed an expression vector combining the promoter together with wheat Na^+/H^+ antiporter (*TaNHX2*), and then transferred it into Nipponbare (Chen, 2007).

Seed germination is usually the most critical stage in seedling establishment because it determines successful crop production (Almansouri et al., 2001; Cheng et al., 2008). Tolerance at emergence is based on survival, whereas tolerance after emergence is based on decrease in growth or yield (Jamil et al., 2007). In addition, rice is considered more sensitive to salts during the early

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seedling period than at the reproductive stages (Sahi et al., 2006). Thus, traits related to germination and early seedling growth could be used to assess saline-alkali tolerance in rice. In this present study, four different concentrations of NaCl and Na_2CO_3 solutions were used to simulate different levels of saline and alkali stresses. The aim of this study was to determine whether transgenic rice has better ability to germinate and more desirable growth traits after germination under stress conditions.

MATERIALS AND METHODS

Seed treatment

Nipponbare (wild type) seeds were obtained from China National Rice Research Institute, Hangzhou, China. T_3-transgenic rice seeds were provided by our laboratory (Chen, 2007). Seeds were surface sterilized in 3% NaOCl solution for 10 min, then rinsed three times with sterilized distilled water. Seeds were blotted dry to remove surface water, then transferred to two sheets of filter paper in Petri dishes, with 30 grains of rice seeds per dish. Both rice varieties were divided into four groups and treated with different concentrations of NaCl (0, 0.5, 1.0 and 1.5%) and Na_2CO_3 (0, 0.1, 0.15 and 0.2%) solutions. Seeds were allowed to germinate in the dark in a growth chamber at 30 ºC for seven days. Before dry weight measurements, rice seedlings were dried in a drying cabinet at 65ºC overnight.

Growth parameters

The number of germinated seeds was counted each day from day 4 to day 7. Seeds were considered to be germinated when their roots/shoots reach the length of 0.4 cm/0.2 cm. Germination percentages of seeds were calculated as follows: Germination (%) = (number of germinated rice seeds in 7 days/total rice seeds) × 100%. A total of 10 seedlings from each variety and treatment were sampled randomly at day 7. Root length and dry weight of seedlings were measured (Cheng et al., 2008).

Design and statistical analysis

The experiment was designed using a randomized complete block design, with five replications for germination percentage and ten replications for growth trait measurements. Analysis of variance was performed using Microsoft excel. Mean values for plant growth traits under different treatments and between varieties were compared through Student-Newman-Keuls test and t-test.

RESULTS

The germination response of transgenic rice and wild type was compared under different sodium salts stress conditions. Saline and alkali stress delayed the germination of rice seeds. In the control group, germination percentage on day 5 was the same as on day 7. However, in other treatment groups, germination percentages continued rising from day 4 to day 7 (data not shown).

When salt concentration increased, the germination rates of both varieties decreased. The wild type reduced more drastically than transgenic rice (Table 1). There was a significant difference between the two types of rice under the same NaCl treatment. Wild type rice has a greater sensitive reception to Na_2CO_3 stress than to NaCl stress (Figure 1). When treated with 0.1% Na_2CO_3 solution, germination percentage of the wild type rice decreased by more than 40%, whereas no change occurred for transgenic rice. When Na_2CO_3 concentration reached 0.2%, the wild type seeds could hardly bear the stress. In contrast, over 60% of transgenic rice seeds were still alive (Table 1).

Further studies were carried out to investigate the effect of NaCl and Na_2CO_3 on early seedling growth of both rice types. Root lengths of both varieties were inhibited with increasing NaCl percentage. Root length was very sensitive to 0.5% NaCl solution. When NaCl reached 1.5%, root length of the wild type was extremely restrained. However, there was a very significant difference in root length between the two varieties when treated with the same percentage of NaCl (Figure 2). Transgenic rice had a more constant root length under increasing NaCl percentage. Dry weight seemed less sensitive to NaCl stress than root length because there was no significant variation under the 0.5% NaCl solution compared with the control. When treated with 1.5% NaCl solution, dry weight of the transgenic rice remained in the same level as that of the control (Table 1).

Root length of both rice types was also suppressed by Na_2CO_3 stress. Obvious differences between the two varieties under all Na_2CO_3 stress levels were observed. Dry weight of the wild type rice gradually decreased with increasing Na_2CO_3 percentage. There were no significant differences on the dry weight of transgenic rice under different concentrations of Na_2CO_3 treatment (Table 1).

DISCUSSION

High salinity and alkalinity cause both hyper ionic and hyper osmotic stresses, which lead to crop water absorption inadequacy and disturbance of poisonous ions exclusion and then plant death. Plant respond to abiotic stresses mainly in three different levels, including physiological, biochemical and molecular responses. These reactions refer to recognition of root signals, decline in net photosynthesis, reduced growth rates, transient decrease in photo chemical efficiency, accumulation of stress matabolites, increase in antioxidative enzymes, reduced ROS (reactive oxygen species) accumulation and stress responsive gene expression (Shao et al., 2008). Unraveling the physiological and molecular basis for the plasticity of rice defending metabolism can get access to stress induced promoter/genes and obtain selection markers for breeding stress resistance varieties, and also ensures

Table 1. Germination percentage (Mean ± SE, n = 5), root length (Mean ± SE, n = 10) and dry weight (Mean ± SE, n = 10) at different levels of different salt solutions in Nipponbare (WT) and transgenic rice (TR).

Test solution and concentration	Germination (%)		Root length (cm)		Dry weight (mg)	
	WT	TR	WT	TR	WT	TR
NaCl						
0	98.8 ± 5.4^{a}	99.0 ± 2.2^{a}	8.99 ± 1.25^{a}	8.16 ± 1.35^{a}	22.08 ± 2.01^{a}	23.21 ± 2.20^{ac}
0.5%	77.5 ± 6.4^{b}	92.0 ± 4.5^{b}	4.28 ± 1.15^{b}	5.89 ± 0.66^{b}	21.14 ± 1.69^{a}	21.45 ± 1.82^{a}
1.0%	56.2 ± 4.2^{c}	64.0 ± 4.2^{c}	3.21 ± 0.72^{c}	5.15 ± 0.56^{b}	16.86 ± 1.19^{b}	19.07 ± 2.25^{b}
1.5%	39.5 ± 6.4^{d}	51.0 ± 4.2^{d}	1.99 ± 0.53^{d}	4.12 ± 0.89^{c}	14.01 ± 1.20^{c}	24.62 ± 3.50^{c}
Na_2CO_3						
0	98.9 ± 10.5^{a}	99.0 ± 2.2^{a}	5.53 ± 0.99^{a}	5.53 ± 0.88^{a}	18.94 ± 1.39^{a}	18.96 ± 2.35^{a}
0.1%	56.5 ± 10.2^{b}	98.0 ± 2.7^{a}	2.91 ± 0.55^{b}	3.90 ± 0.73^{b}	17.88 ± 0.82^{b}	17.93 ± 1.86^{a}
0.15%	59.7 ± 4.3^{b}	90.0 ± 3.5^{b}	2.29 ± 0.61^{c}	2.85 ± 0.60^{c}	16.10 ± 0.97^{c}	18.04 ± 1.84^{a}
0.2%	25.1 ± 6.6^{c}	65.0 ± 3.5^{c}	1.49 ± 0.26^{d}	1.92 ± 0.49^{d}	14.18 ± 1.13^{d}	17.45 ± 1.68^{a}

Different letters indicate significant differences from different concentrations in same salt treatment ($p < 0.05$).

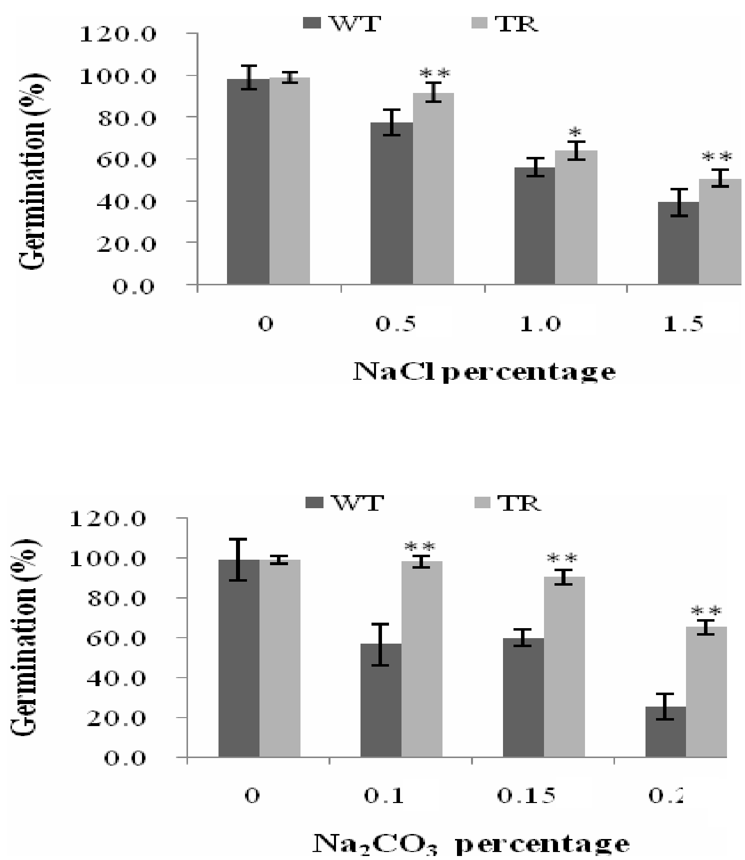

Figure 1. Effect of NaCl and Na_2CO_3 on germination in Nipponbare (WT) and transgenic rice (TR). *<0.05, **<0.01.

improved food security and improving ecoenvironmental construction worldwide.

Transgenic breeding is a good way to improve crop tolerance of abiotic stresses. Li et al. (2010) transferred a *Thioredoxin (Trx)* gene into barley and found that the transgenic barley with overexpressed *PTrx* activates protective responses to aluminum stress during germination. Recently, many transgenic rice materials with salt

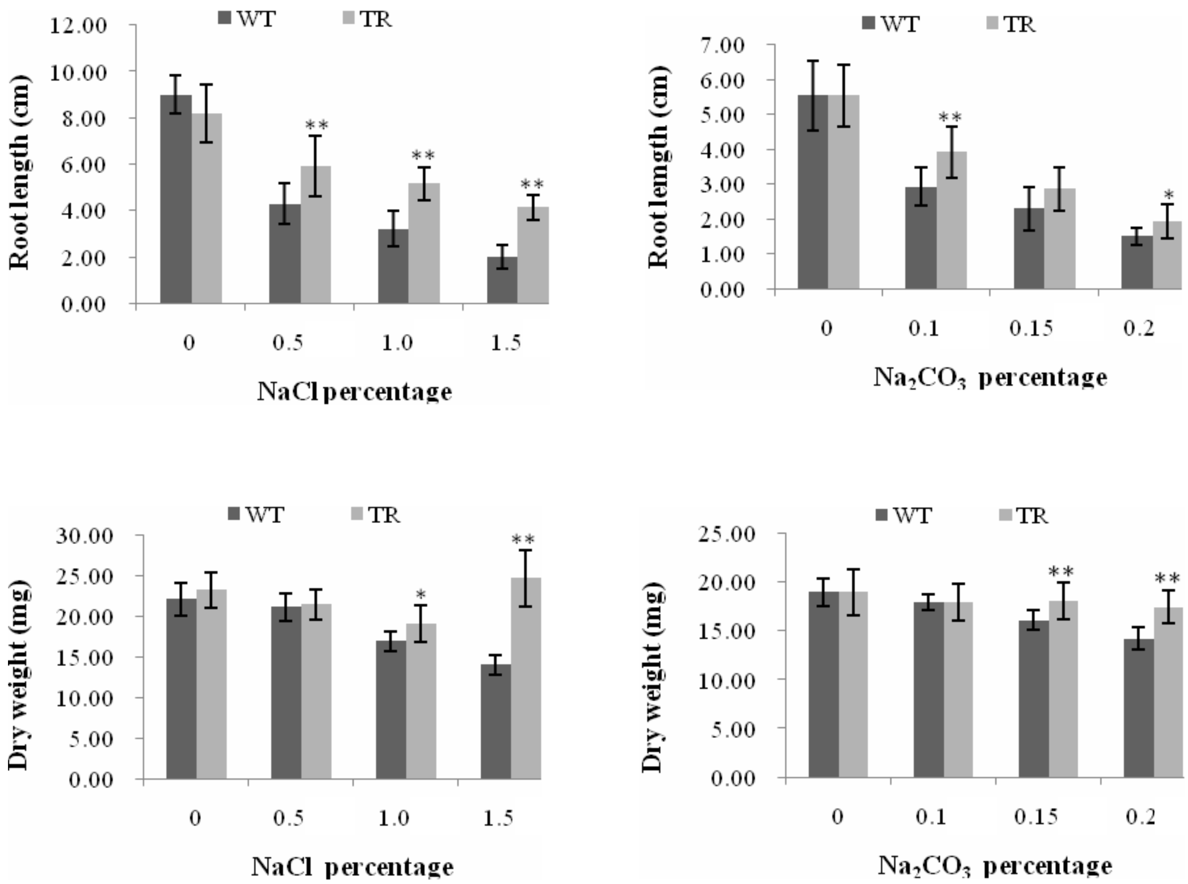

Figure 2. Effect of NaCl and Na_2CO_3 on root length and dry weight in wild type (WT) and transgenic rice (TR). *<0.05, **<0.01.

tolerance have been obtained (Sahi et al., 2006). The detailed dissections of the regulatory networks that govern higher plants' responses to abiotic and biotic stress have been studied almost exclusively in controlled environments where a single challenge has been applied. However, there were few reports on both saline and alkali tolerance in transgenic rice. In the present study, saline-alkali tolerance of the wild type rice and its transgenic variety were compared. Previous research showed that rice has an acute sense of saline-alkali stress, especially alkali stress with high pH value (Gao et al., 2010). As mentioned above, the germination seedling stage was the most vulnerable period to saline-alkali stress, so the study of a few traits related to saline-alkali stress in this stage can give insights into plasticity of transgenic rice before and after seedling stage under such stress. In the current study, saline-alkali tolerance during seed germination was measured on germination percentage. Saline-alkali tolerance during seedling growth was also assessed on the basis of root length and dry weight at a given sodium salt percentage. Considerable variations were determined in seed germination and early seedling growth between the wild type and its transgenic variety. Germination percentage of transgenic rice is higher than wild type under both NaCl and Na_2CO_3 stress conditions. When treated with NaCl or Na_2CO_3 solutions, root length of both varieties was inhibited with increased concentration of salts. Wild type rice was definitely more sensitive to sodium salts than the transgenic variety. The results was in accordance with Li's (2010) research finding that barely root growth were seriously inhibited after Al^{3+} treatment. Dry weight was a less varying trait than the others. When NaCl or Na_2CO_3 concentration was increased, dry weight of wild rice declined accordingly. However, there were no dominant decreases in dry weight of transgenic rice. This was especially true when treated with different Na_2CO_3 solutions. Dry weight of transgenic type even increased considerably when treated with 1.5% NaCl solution compared with the control. Material synthesis within plant was more active in transgenic rice than in wild rice under NaCl or Na_2CO_3 stress. This supported the claim of the former reports which photosynthetic apparatus and membrane could be affected by water stress (Shao et al., 2008).

In conclusion, the transgenic rice with the expression vector of 796 bp promoter and *TaNHX2* has better properties to resist saline-alkali stress compared with the wild type. These properties keep rice seeds with high germination percentage when soaked in salt solutions. Root growth is also less inhibited by over expressing the Na^+/H^+ antiporter; this contributes considerably to rice growth and development. The Na^+/H^+ antiporter activates V-ATPase, V-PPase, and P-ATPase, which associate together to provide enough electrochemical power to pump Na^+ from cytoplasm to vacuole, thus rendering transgenic rice more tolerant to severe stress from salts (Qiu et al., 2004). It is possible that, in the near future, *TaNHX2*-overexpressed rice could grow in saline-alkali soil without any production reducing because of the enhanced saline-alkali resistance.

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