

EFFECTS OF SALT STRESS ON ABSORPTION AND DISTRIBUTION OF OSMOTIC IONS IN WHEAT SEEDLINGS

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Abstract

Effects of salt stress on absorption and distribution of osmotic ions in wheat seedlings were studied with 4 winter wheat varieties under NaCl stress by exploring dynamic changes of Ca²⁺, K⁺, Na⁺, Ca²⁺/Na⁺, K⁺/Na⁺ and Na⁺ limiting ability. The results showed that the contents of K⁺, Ca²⁺ and the K⁺/Na⁺ ratio gradually decreased in a manner of both salt concentration dependent and stress time dependent. The extent of reduction of K⁺ and Ca²⁺ in root was much more severe than that in shoot. The ability of Na⁺ limiting in QM6 and DK961 was stronger than that in JM22 and QF1. These results indicate that the maintenance of higher Na⁺ content in root, Na⁺ limiting ability, Ca²⁺ and K⁺ concentration will be the main physiological traits for the salt-tolerance of wheat.

Salinity, one of the main environmental factors affecting crop yield, can reduce crop yield by more than 40% (Liu *et al.* 2008). The high concentration of Na⁺ in the cytoplasm will destroy the stability of protein structure, inhibit the activity of the enzyme, and even lead to cell death (Amtmann *et al.* 1998). For example, the excessive accumulation of Na⁺ inhibits the absorption of K⁺, Ca²⁺ and so on, which results in the inhibition of growth, poor development, and even death (Zhang 2007).

High concentration of K⁺ in epidermal and guard cells is necessary for regulation of stomata opening and closure under salt stress (Tavakkoli *et al.* 2011). An important impact of salt stress on plant growth is to induce ion imbalance in cells, such as lower concentrations of K⁺, which results in ion (Na⁺) toxicity (Song and Fujiyama 1996). Therefore, reducing Na⁺ loading into the xylem and maintaining higher K⁺/Na⁺ are the most effective ways for using salty soils (Cuin *et al.* 2003).

Calcium (Ca²⁺), one of the important ion to eliminate and relieve wheat salt stress, can improve seed germination ability and maintain the stability of cytoplasmic membrane and cytoderm under salt stress. Ca²⁺ can also interact with calmodulin (CaM) to participate in many physiological metabolic processes (Li *et al.* 2006, Zhao *et al.* 2006). Therefore, understanding the mechanisms of Na⁺, K⁺, Ca²⁺ distribution, K⁺/Na⁺ and Na⁺ limiting ability of wheat seedlings is essential to improve wheat salt tolerance.

The selected four wheat (*Triticum aestivum* L.) cultivars seeds, were Jimai22 (JM22) (collected from Shandong academy of agricultural sciences), Qingmai6 (QM6) (collected from Qingdao Agricultural University), Qingfeng1 (QF1) (collected from Shandong qingfeng seed co. Ltd.) and Dekang961 (DK961) (collected from Dezhou academy of agricultural sciences). Thirty seeds of each cultivar were rinsed in distilled water for 24 hrs at 25°C (in order to keep consistent germination) in a thermostat and then were cultured by half strength Hoagland's nutrient solution

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(pH 6.0, 2019) for one week. Then the seven-day old seedlings were irrigated with Hoagland nutrient solution (pH 6.0) for another seven days at 22°C/18°C (day/night) and 16 hrs/8 hrs (light/dark) with root aeration. The seedlings with three leaves (2.5 g seedlings/cultivar were taken) were flushed with distilled water to leach out the nutrient liquid and then were cultured by 0, 80, 160 and 240 mmol NaCl (prepared in Hoagland solution, pH 6.0). For each treatment, there were three replications. Ten wheat seedlings were extracted randomly to measure the content of Na⁺, K⁺, Ca²⁺ after treated for 0, 1, 4 and 7 days, respectively.

The samples were dried in an oven at 80°C (Bao 2007) for 48 hrs before the analysis of ions content. After drying, the shoots and roots were ground into powder, respectively. The major inorganic ions (Na⁺, K⁺, Ca²⁺) were analyzed using an atomic absorption spectrometer (Bao 2007). Na⁺ limiting ability in the sample was estimated using following formula (Wang *et al.* 2011): Na⁺ limiting ability = Na⁺ content of root/Na⁺ content of shoot statistical analysis was performed using SPASS 19.0.

Ca²⁺ content of wheat shoot and root gradually decreased under salt stress, and the greater decrease was found in shoot (Fig. 1). After 7 days treatment, calcium concentrations decreased roughly linearly under the control condition (unstressed). The four genotypes responded differently under lower Na⁺ concentration (80 and 160 mmol) treatment for 4 and 7 days and QF1 had the least Ca²⁺ content compared with the other three cultivars. The shoots and roots had higher content of Ca²⁺ in QM6 and DK961 compared with JM22 and QF1. Compared with control, the content of Ca²⁺ in root was reduced by 50.11% in JM22, 46.58% in QM6, 45.03% in DK961 and 58.96% in QF1 respectively in 240mmol saline solution for 7d. Salt solution also reduced the Ca²⁺ concentrations of root to 58.46, 64.08, 62.93 and 48.64% in JM22, QM6, DK961 and QF1, respectively, compared to that in control group. Ca²⁺ can improve salinity tolerance, especially in sensitive genotypes (Rengel 1992 and Genc *et al.* 2010). Thus, from the practical point of view, higher Ca²⁺ concentration may be an important trait for salinity breeding programs.

The K⁺ concentration of wheat root and shoot decreased gradually along with increment of external salinity, but the extent in shoot was smaller than in root (Fig. 2). Under normal condition, the content of K⁺ did not display significant changes among the four genotypes in the course of the treatment. Otherwise, the shoot maintains higher K⁺ concentrations than root under 80 and 160 mmol NaCl treatment. After 7 days of higher salt stress treatment (160 and 240 mmol), the K⁺ concentration of root reduced dramatically to 30 mg/g and below. In JM22 and QF1, K⁺ content decreased more rapidly when compared with QM6 and DK961 as salt concentration increased and time went on. K⁺ concentration in shoot was reduced by 48.75% in JM22, 39.88% in QM6, 39.15% in DK961 and 46.67% in QF1, respectively compared to control in 240 mmol saline solution for 7 days. In wheat, it is well known that K⁺ homeostasis is very important for salt tolerance (Dvor *et al.* 1994). Maintenance of high K⁺ concentration is one of the mechanisms underlying salt tolerance (Britto *et al.* 2010). The present results are consistent with previous studies, since the content of K⁺ was higher in salt-tolerant varieties QM6 and DK961 than salt-sensitive varieties JM22 and QF1 (Fig. 2).

As shown in Fig. 3, the content of Na⁺ increased sharply with salt stress treatment, but it did not differ significantly among tested cultivars in non-saline solution. Compared with JM22 and QF1, the Na⁺ concentration in shoot increased less in QM6 and DK961 (Fig. 3A). Meanwhile, after 7d treatment with 160 mmol salt stress, the content of Na⁺ in shoot was 8.4-fold in QM6 and 17.27-fold in JM22 compared to control, respectively. In root, compared with the other three genotypes, the Na⁺ concentration of QF1 was the lowest under 160 mmol salt stress Na⁺ (Fig. 3B). Otherwise, the content of Na⁺ was 13.75-folds in JM22, 15.35-folds in QM6, 15.86-folds in DK961 and 13.62-folds in QF1 under 240 mmol salt stress treatment for 7 days compared to

control. Shi *et al.* (2014) found that wheat shoot had higher Na^+ concentration than root under high salt treatment. The present results are consistent with this findings.

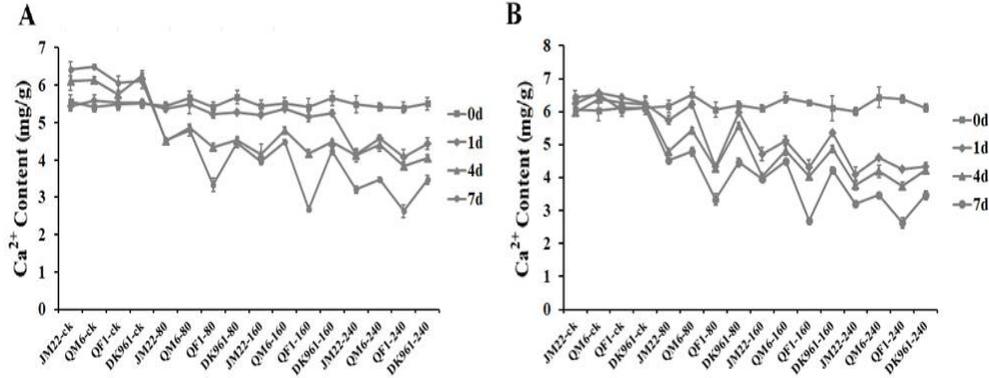


Fig. 1. Dynamic changes of Ca^{2+} content in different tissues of wheat seedlings under salt stress treatments. (A) shoot; (B) root.

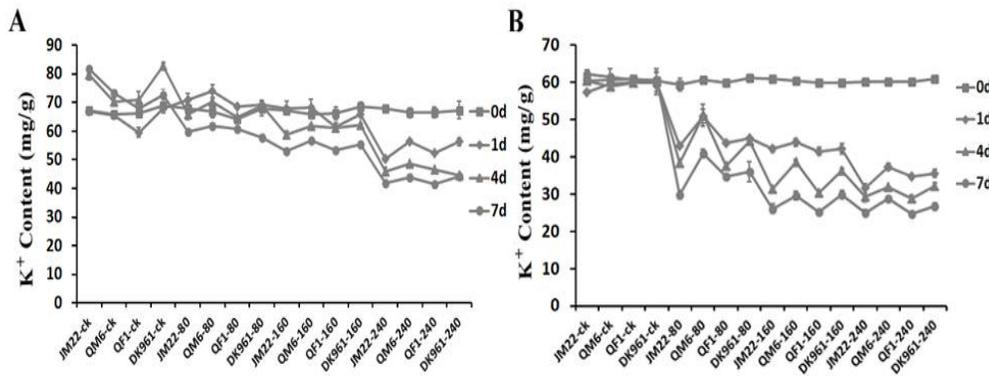


Fig. 2. Effects of different salt stress treatments on the dynamic changes of K^+ content in winter wheat. (A) shoot; (B) root.

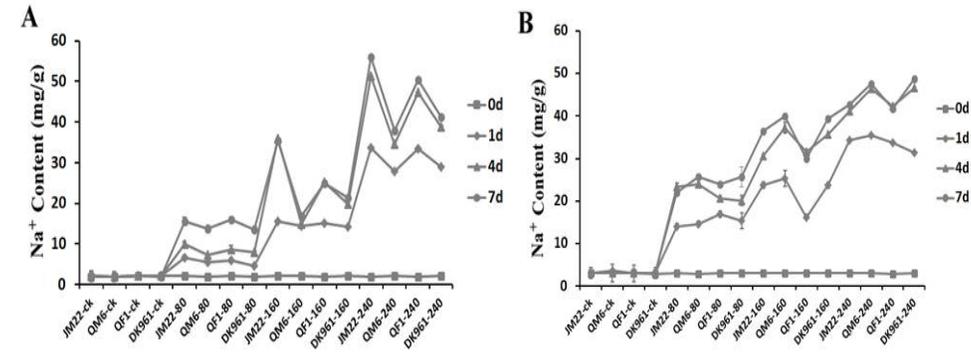


Fig. 3. Effects of different salt stress treatments on the dynamic changes of Na^+ content in winter wheat. (A) shoot; (B) root.

Compared with control, the $\text{Ca}^{2+}/\text{Na}^{+}$ of wheat shoot and root declined steeply under salt stress, and the extent increased gradually with the increase of salt concentration increasing and time prolonging (Fig. 4). Under normal condition, the $\text{Ca}^{2+}/\text{Na}^{+}$ did not differ significantly. The $\text{Ca}^{2+}/\text{Na}^{+}$ of shoot in QM6 and DK961 decreased slight compared with JM22 and QF1 under salt stress (Fig. 4A), and the same tendency was found in root (Fig. 4B). The $\text{Ca}^{2+}/\text{Na}^{+}$ of root in QM6, DK961 and JM22 decreased more compared to QF1 under salt stress, but the difference was narrow.

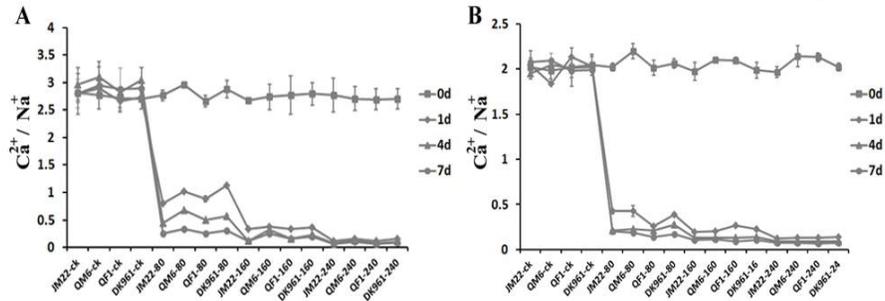


Fig. 4. The $\text{Ca}^{2+}/\text{Na}^{+}$ of wheat shoot (A) and root (B) in QM6, DK961, JM22 and QF1 under different salt stress treatments over 7 days.

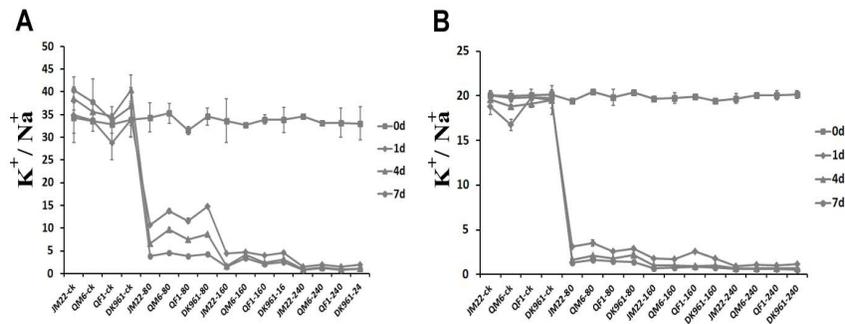


Fig. 5. The $\text{K}^{+}/\text{Na}^{+}$ of wheat shoot (A) and root (B) in QM6, DK961, JM22 and QF1 under different salt stress treatments over 7 days.

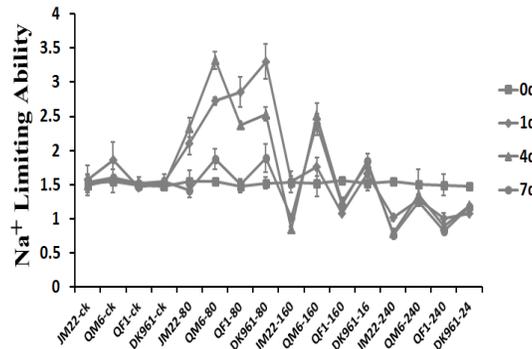


Fig. 6. The Na^{+} limiting ability of wheat shoot (A) and root (B) in QM6, DK961, JM22 and QF1 under different salt stress treatments over 7 days.

Similarly, $\text{Ca}^{2+}/\text{Na}^{+}$, the $\text{K}^{+}/\text{Na}^{+}$ declined sharply under salt stress although it did not differ significantly under non-saline solution (Fig. 5). But the $\text{K}^{+}/\text{Na}^{+}$ of shoot in QM6 and DK961 was higher than in JM22 and QF1 under salt stress. The $\text{K}^{+}/\text{Na}^{+}$ of QM6 was 3.39 under 160 mmol salt stress treatment for 7 days, which was necessary for ion balance and maintaining high salt tolerance (Fig. 5A). The same changing tendency of $\text{K}^{+}/\text{Na}^{+}$ was found in root under salt stress, but the difference was less and differences among varieties were not significant neither (Fig. 5B).

Na^{+} limiting ability was almost the same under normal condition, but the changing tendency displayed significant difference after salt stress treatment (Fig. 6). After 80 mmol NaCl treatment, Na^{+} limiting ability was higher at 1 and 4 days than at 7 days, and Na^{+} limiting ability of JM22 and QF1 was lower than QM6 and DK961. It was speculated that the lower Na^{+} concentration improved Na^{+} limiting ability of wheat. The Na^{+} limiting ability was higher in QM6 and DK961 under 160 mmol salt stress treatment compared to control, which suggested that they had higher salt tolerance. The Na^{+} limiting ability of the four genotypes was lower than control under 240 mmol salt stress treatment, and the extent was greater in JM22 and QF1 than QM6 and DK961. Under 240 mmol salt stress treatment for 7 days, Na^{+} limiting ability reduced to 49.61, 78.04, 75.90 and 54.28% in JM22, QM6, DK961 and QF1, respectively. All of these results indicated that the Na^{+} limiting ability was higher in QM6 and DK961, and it differed significantly compared to non-saline solution.

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