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Full Length Research Paper

Na^+ localization and re-transportation of buckwheat seedlings

Zhan Wei-Yan[1], Zhou Gong-Ke[2] and Yang Hong-Bing[1]*

[1]College of Life Science, Qingdao Agricultural University, Qingdao 266109, China.
[2]Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, Qingdao 266101, China.

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Salt-tolerant buckwheat (*Fagopyrum esculentum* Moench) variety Chuanqiao No.1 and salt-sensitive buckwheat variety TQ-0808 were used as experimental materials. Na^+ content of roots, stem base and leaves were determined to find out the localization and re-transportation traits of buckwheat under NaCl stress and removing NaCl stress. It showed that Na^+ accumulation of roots and stem base of salt-tolerant buckwheat variety was obviously more than that of the salt-sensitive one, which could efficiently restrict Na^+ from transporting to shoot, and made salt-tolerant buckwheat variety has less Na^+ content of leaves and more salt tolerance. Na^+ accumulation capability of stem base was obviously higher than that of roots of the two buckwheat varieties; it showed that stem base was a very important Na^+ exclusion localization of buckwheat. Na^+ re-transportation capability of leaves of salt-tolerant buckwheat variety was obviously higher than that of the salt-sensitive one after removing NaCl stress, which had more correlation with higher Na^+ re-transportation capability of salt-tolerant buckwheat variety. In addition, the net photosynthetic rate of salt-tolerant buckwheat variety had not obviously changed in contrast with control under NaCl stress; however, that of salt-sensitive buckwheat variety significantly decreased, and it was still significantly lower than the control after removing NaCl stress. These findings showed that the part of roots and stem base of buckwheat might be the main Na^+ exclusion localization.

Key words: Buckwheat, NaCl stress, Na^+ localization, re-transportation.

INTRODUCTION

Currently there is a large area of salinized land in China, 2.7×10^{11} m^2 is taken over by salinized and secondary salinized land (Zhou et al., 2009). The mechanisms of salt tolerance in plants mainly include salt-secretion, salt-dilution, salt-sedimentation, salt-exclusion, K^+/Na^+, structure and function of membrane, ion localization, osmotic adjustment, hormone adjustment, mechanism of scavenging reactive oxygen in cells, salt-induced protein and so on (Liu et al., 1987). Researches showed that adaptive types of salt stress mainly included salt-dilution, salt-excretion and salt-exclusion in plants. Halophytes are generally salt-dilution and salt-excretion types, glycolphytes are usually salt-exclusion types (Liu et al., 1987).

Reducing salinity of shoot, especially that of leaves, and keeping the function of photosynthesis were important mechanisms of salt-tolerance in plants (Liu et al., 1987; Greenway and Munns, 1980). Buckwheat (*Fagopyrum esculentum Moench*) is a dicotyledonous polygonaceae plant which has high nutrient value, good medical treatment health care and developmental potential (Wang et al., 2003). Buckwheat is not only an ideal raw material for health, but also an ideal nutrition food.

Nowadays, with improving of people's material and cultural living standard, people attach great importance to health food and diet, then comprehensive utilization researches of buckwheat spring up like mushrooms; meanwhile, the stress resistance of buckwheat is strong, especially in drought and infertile soil, and the patience to salt stress is higher than other food crops (Zhang and Chen, 2004). So more and more researches were investigated with buckwheat. Early studies showed that

*Corresponding author. E-mail: hbyang@qau.edu.cn. Tel: +86-532-88030487.

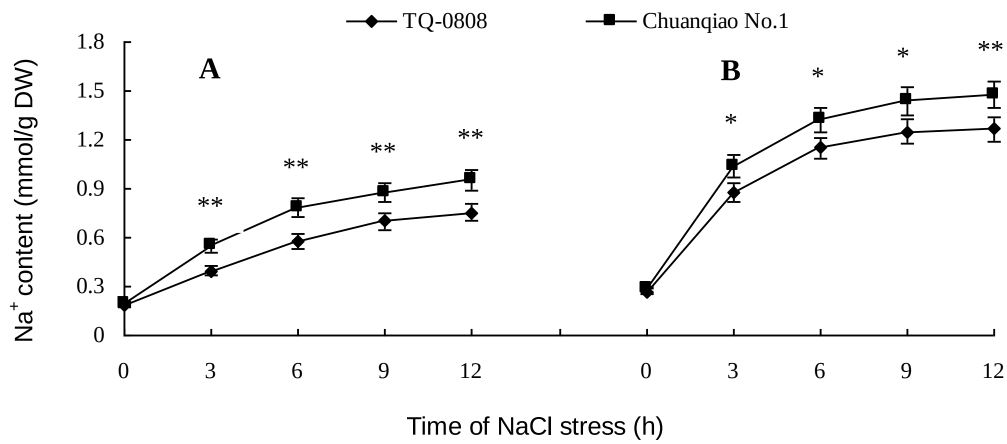

Figure 1. The time course of Na^+ absorption of roots (A) and stem base (B) of buckwheat seedlings under NaCl stress. Data are mean ± SE (n = 3, *P < 0.05; ** P < 0.01).

salt-tolerant buckwheat variety Chuanqiao No.1 accumulated more Na^+ in roots and stem base, while salt-sensitive one TQ-0808 mainly accumulated Na^+ in roots, and the quantity of accumulation in TQ-0808 was obviously lower that in Chuanqiao No.1. Na^+ restriction capability of salt-tolerant buckwheat variety was higher than that of the salt-sensitive one, which made salt-tolerant buckwheat variety to have less Na^+ content of leaves and more salt tolerance (Ma et al., 2009a). Previous experiments showed that salt-sensitive variety TQ-0808 could not bear for a longer period of time under 100 mmol/L NaCl stress, but it was stable in not more than 12 h. In this paper, two buckwheat varieties with difference in salt-tolerance were selected in previous experiments and were used as experimental materials, and the distribution and re-transportation traits of buckwheat were studied through comparison of Na^+ content under NaCl stress and removing NaCl stress.

MATERIALS AND METHODS

Plant material cultivation and stress treatments

Salt-tolerant buckwheat variety Chuanqiao No.1 and salt-sensitive buckwheat variety TQ-0808 selected in previous experiments were used as experimental materials (Ma et al., 2009b). Selecting grain size and full buckwheat seeds, using 1 g/L $KMnO_4$ for seed disinfection for 10 min, then seeds were cultivated in an incubator at 26°C. When seeds germinated, seedlings were removed into plastic basins in growth chambers and cultivated with Hoagland nutrient solution, nature light, day and night temperature were 26 and 16°C, relative humidity was 60%, common managed. Seedlings were treated with NaCl (NaCl solutions were confected with Hoagland nutrient solution) at the stage of 10 cm height. The concentration of NaCl was 100 mmol/L, separately took roots, stem base and leaves at 3, 6, 9 and 12 h. Killed materials in oven (FY-DR-2) at 105°C for 10 min, then dried materials to constant weight at 80°C. Besides, seedlings were treated with 100 mmol/L NaCl for 9 h and then washed roots with deionized water to remove NaCl stress, then removed them into Hoagland solution, and took materials at 6, 12, 18 and 24 h separately. Materials were treated with the same methods and each treatment was 3 replicates.

Determination of Na^+ content

10 mg of materials were put into crucibles and incinerated for 20 h at 500°C. Crucibles were taken from the furnace, cooled to room temperature and 1 to 2 drop(s) of concentrated nitric acid were added to dissolve ash. 10 mL of deionized water was added. Na^+ content was measured with atomic absorption spectrophotometer (AA2600).

Determination of net photosynthetic rate

Seedlings were cultivated as before and were treated with 100 mmol/L NaCl (NaCl solution were confected with Hoagland nutrient solution) for 9 h at the stage of 10 cm height, and used CIRAS-I portable photosynthesis determination systems (Haverhill, Massachusetts, USA) to determine the net photosynthetic rate of buckwheat leaves, then washed roots with deionized water to remove NaCl stress, and removed them into Hoagland solution. After 24 h, the same method was used to measure the net photosynthetic rate of buckwheat leaves.

Statistical analysis

Student's t-test was used for comparison between different treatments. A difference was considered to be statistically significant when P < 0.05, and especially significant when P < 0.01 (Wang et al., 2010).

RESULTS

The time course of Na^+ absorption of roots and stem base of buckwheat seedlings under NaCl stress

With the increase of stress time, Na^+ content of roots and stem base of the two buckwheat varieties showed obvious accumulation tendency (Figure 1). The quantity

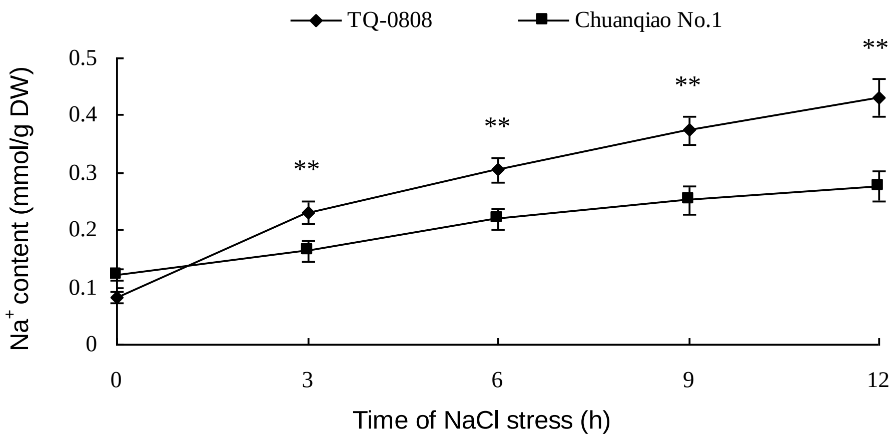

Figure 2. Effect of NaCl stress on Na^+ content of leaves of buckwheat seedlings. Data are mean ± SE (n = 3, *P < 0.05; ** P < 0.01).

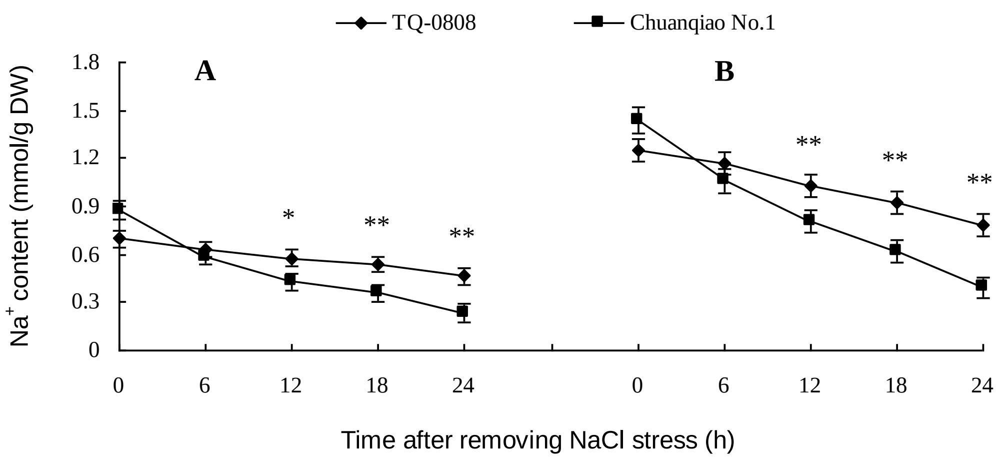

Figure 3. Na^+ re-transportation of roots (A) and stem base (B) of buckwheat seedlings after removing NaCl stress. Data are mean ± SE (n = 3, *P < 0.05; **P < 0.01).

of Na^+ accumulation of Chuanqiao No.1 was obviously more than that of TQ-0808. The accumulation of Na^+ was stable at 9 h. From the whole effect of Na^+ accumulation, the quantity of Na^+ accumulation of roots of Chuanqiao No.1 was 1.33 folds as that of TQ-0808, and that of stem base of Chuanqiao No.1 was 1.20 folds as that of TQ-0808. In addition, the quantity of Na^+ accumulation of stem base of the two buckwheat varieties was obviously more than that of roots. When the accumulation of Na^+ was stable, the quantity of Na^+ accumulation of stem base of TQ-0808 was 1.91 folds as that of its roots, and that of the stem base of Chuanqiao No.1 was 1.69 folds as that of its roots.

Effect of NaCl stress on Na^+ content of leaves of buckwheat seedlings

With the increase of stress time, Na^+ content of leaves of the two buckwheat varieties showed obvious accumulation tendency (Figure 2). Na^+ content of leaves of TQ-0808 increased more than that of Chuanqiao No.1. After 3 h, Na^+ content of TQ-0808 was obviously higher than that of Chuanqiao No.1. Na^+ content of leaves of TQ-0808 was 1.56 folds as that of Chuanqiao No.1 at 12 h. Seeing from the total Na^+ accumulation of leaves, the quantity of Na^+ accumulation of TQ-0808 was 2.25 folds as that of Chuanqiao No.1, so TQ-0808 accumulated obviously more quantity of Na^+ than Chuanqiao No.1 did

Na^+ re-transportation of roots and stem base of buckwheat seedlings after removing NaCl stress

As shown in Figure 3, Na^+ content of roots and stem base of the two buckwheat varieties decreased with the increase of time of removing NaCl stress, and Chuanqiao

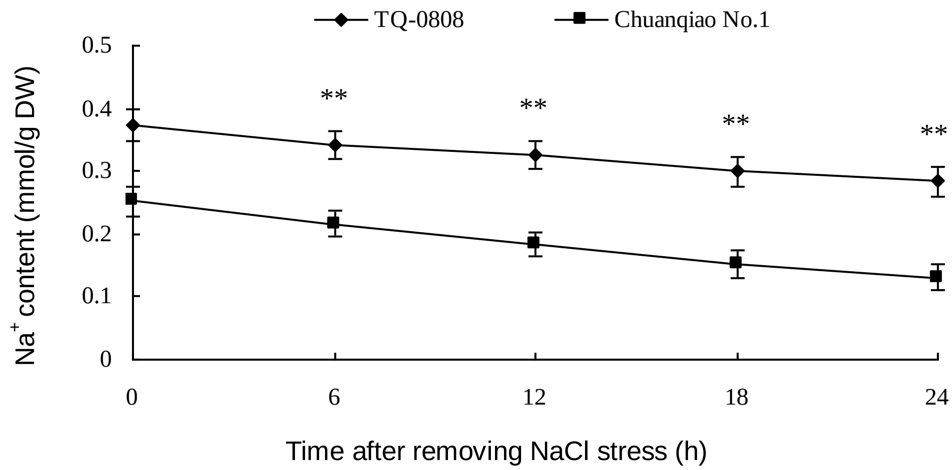

Figure 4. Na^+ re-transportation of leaves of buckwheat seedlings after removing NaCl stress. Data are mean ± SE (n = 3, *P < 0.05; ** P < 0.01).

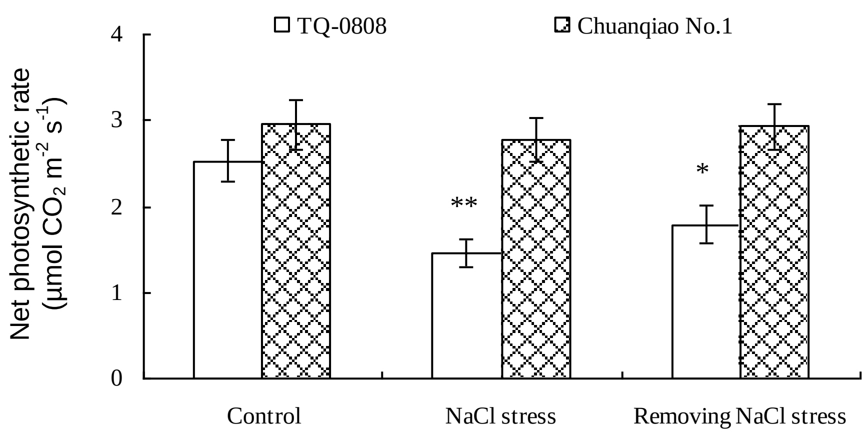

Figure 5. Net photosynthetic rate of buckwheat seedlings under NaCl stress and removing NaCl stress. Data are mean ± SE (n = 3, *P < 0.05; **P < 0.01).

No.1 decreased more quickly. Na^+ content of roots and stem base of Chuanqiao No.1 was obviously lower than that of TQ-0808 at 12 h. Na^+ content of Chuanqiao No.1 was close to control at 24 h. Seeing from the total quantity of Na^+ re-transportation, that of roots of Chuanqiao No.1 was 2.72 folds as that of TQ-0808, and that of stem base of Chuanqiao No.1 was 2.24 folds as that of TQ-0808. The capability of Na^+ re-transportation of roots and stem base of Chuanqiao No.1 was higher than that of TQ-0808.

Na^+ re-transportation of leaves of buckwheat seedlings after removing NaCl stress

With the increase of time of removing NaCl stress, Na^+ content of leaves of the two buckwheat varieties showed a decrease tendency (Figure 4), and that of Chuanqiao No.1 decreased more than that of TQ-0808. Na^+ content of leaves of Chuanqiao No.1 was close to control at 24 h. Seeing from the total quantity of Na^+ re-transportation of leaves, that of Chuanqiao No.1 was 1.37 folds as that of TQ-0808. So the capability of Na^+ re-transportation of leaves of Chuanqiao No.1 was higher than that of TQ-0808.

Net photosynthetic rate of buckwheat seedlings under NaCl stress and removing NaCl stress

Net photosynthetic rate of leaves of Chuanqiao No.1 had not obvious change in contrast with control under NaCl stress, while that of TQ-0808 significantly decreased (Figure 5). After removing NaCl stress, net photosynthetic rate of leaves of TQ-0808 increased to some extent, but it was still significantly lower than the control.

DISCUSSION

Salt tolerance of glycophyte mainly depends on the selective absorption of ions of roots and localization of salt in organ, tissue and cell levels (Epstein et al., 1983). In the fulfillment of cell osmoregulation and the need of ion, plants could maintain comparatively lower salinity (Pitman, 1984). Apple stock M26 with high salt tolerance could retain Na^+ in roots to restrict Na^+ from transporting to leaves (Dinkelbury and Ludders, 1990). Salt-tolerant wheat and rice accumulated the main body of Na^+ in roots and stem base, and less Na^+ content in shoot (Yang et al., 2002; Wang et al., 2008). In this paper, Na^+ accumulation of roots and stem base of salt-tolerant buckwheat variety Chuanqiao No.1 was obviously more than that of the salt-sensitive one TQ-0808, which could effectively restrict Na^+ from transporting to shoot, and made Chuanqiao No.1 obviously to have a lower Na^+ content in leaves than TQ-0808 and more salt tolerance.

In addition, Na^+ accumulation capability of stem base of the two buckwheat varieties was obviously higher than that of roots. This result is consistent with the study of Yu et al. (2003) on wild soybean N23232, whereas opposite to the research of Yang et al. (2004) about *Malus zumi*. It indicated that there was some difference of Na^+ accumulation capability between herbaceous plant and woody plant of the main Na^+ exclusion site. Meanwhile, it also indicated that the stem base of buckwheat was a very important Na^+ exclusion site. Studies indicated that most of the Na^+ absorbed by the shoot of wild soybean BB52 with salt-tolerance were re-transported to roots and the rootstock section after removing NaCl stress, and the Na^+ content of leaves was very low (Yu et al., 2003). Most of the Na^+ of shoot of salt-tolerant reed were re-transported to roots, while salt-sensitive rice re-transported less (Matsushita and Matoh, 1991). This paper also showed that Na^+ re-transportation capability of leaves of salt-tolerant buckwheat variety Chuanqiao No.1 was obviously higher than that of the salt-sensitive one TQ-0808 after removing NaCl stress, and the higher Na^+ re-transportation capability of leaves of Chuanqiao No.1 had more correlation with higher Na^+ re-transportation capability of its main Na^+ exclusion site (roots and stem base).

Meanwhile, photosynthesis of plants is an extremely important metabolic process, normal physiological function of chloroplasts need certain concentration of Na^+ and Cl^- (Wang, 2005), when salinity of soil is beyond certain extents, photosynthetic capacity of plants dropped, higher salinity and more time of operation, it declined more clearly (Munns, 1993). With the increase of time and concentration of the NaCl stress, the content of Na^+ and Cl^- of leaves of salt-tolerant rice and chloroplasts was lower than that of the salt-sensitive one, and the content of Na^+ and Cl^- was clearly negatively correlated with net photosynthetic rate of leaves (Wang et al., 2002). This is the reason why lower salt salinity promoted photosynthetic rate of Chuanqiao No.1 and high salinity showed inhibitory action on TQ-0808 in this experiment. In fact, transportation and localization of Na^+ involved gene expression and regulation, and people had found two genes correlated with Na^+ localization and Na^+ extrusion in mode plant *Arabidopsis*, which lied on tonoplast and plasmolemma and encoded NHX1 and SOS1 Na^+/H^+ antiporter (Serrano and Rodriguez-Navarro, 2001). Therefore, the molecular mechanism of salt-tolerance of buckwheat needed further research through molecular biology methods.

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