DOI: 10.32615/bp.2020.051 BIOLOGIA PLANTARUM 64: 485-489, 2020

Distribution of Na^+ in roots and stem bases of buckwheat seedlings

W.-Y. ZHAN, Y.-C. YU, L.-X. HOU, C.-Y. LIU, F.-G. ZHAO, Y.-P. ZHANG, and H.-B. YANG*

Key Laboratory of Plant Biotechnology, University of Shandong Province,
College of Life Sciences, Qingdao Agricultural University, Qingdao, 266109, P.R. China

Abstract

The localizations of sodium exclusion are roots and stem base, however, Na^+ distribution in these localizations is unclear. Here, we used a salt-tolerant buckwheat cultivar Chuanqiao No.1 and a salt-sensitive cultivar TQ-0808 to demonstrate Na^+ distribution. We found that Na^+ content was highest in vacuole, the following was in cell wall or free space, and the least was in cytoplasm. Comparative analysis shows that Na^+ accumulation in vacuole, cell wall, or free space of roots and stem base in 'Chuanqiao No.1' was obviously higher than in 'TQ-0808'; in contrast, Na^+ accumulation in cytoplasm of 'Chuanqiao No.1' was less than in 'TQ-0808'. These results indicate that the capabilities of Na^+ extrusion and Na^+ compartmentalization of salt-tolerant buckwheat were obviously higher than of the salt-sensitive one, and the capabilities could effectively restrict Na^+ transport to shoot. Compartmentalization of Na^+ in the vacuole was the main way for Na^+ exclusion of salt-tolerant buckwheat. In addition, the transcriptions of *Na^+/H^+ antiporter 1* and *salt overly sensitive 1* were remarkably higher in 'Chuanqiao No.1' than in 'TQ-0808', which is consistent with the above results.

Additional key words: *Fagopyrum esculentum*, salt tolerance, TEM, transcription *NHX1* and *SOS1*.

Introduction

Roots are the crucial parts of plants to absorb or exclude Na^+, and the process of Na^+ transport from the soil solution into the epidermal and cortical cells of plant roots and *vice versa* is controlled by concentration difference or electric potential difference of Na^+. The net accumulation of Na^+ in roots is the result of homeostasis between passive influx and active efflux of Na^+. Restricting Na^+ transport to the shoot of salt-tolerant plants is closely related to the retention of Na^+ in roots (Nie *et al.* 2008), meanwhile, Na^+ extrusion from the original absorption point of roots is also considered as an important mechanism for reducing the injuries of salt stress (Schubert and Läuchli 1990). Salt exclusion from plants in main Na^+ exclusion localizations restrict salt transport to the plant. Compared with the salt-sensitive rice, the stem base of salt-tolerant reed can restrict Na^+ transport to shoot (Matsushita and Matoh 1991). The main Na^+ exclusion localization of wheat has been found in roots and root-stem junction (Yang *et al*. 2001), and in Malus in roots and stem base (Yang *et al*. 2004).

Buckwheat (Fagopyrum esculentum Moench) is a dicotyledonous plant of family Polygonaceae, which has strong salt resistance and wide adaptability (Wang and Li 2004), and it is also economically important (Wei *et al*. 1995). It is reported that the main Na^+ exclusion places of salt-tolerant buckwheat are in roots and stem bases (Ma *et al.* 2011), and capabilities of Na^+ accumulation in roots and Na^+ exclusion in salt-tolerant buckwheat cultivars are obviously higher than in salt-sensitive ones under salinity (Zhan et al. 2011). However, salt exclusion mechanism of buckwheat is not clear yet. Therefore, in this study, salt-tolerant buckwheat 'Chuanqiao No.1' and salt-sensitive 'TQ-0808' were used as experimental materials. Using Na^+ fluorescent localization, Na^+ cytological localization, and energy spectrum analysis, the Na^+ distribution in tissues and cells in main Na^+ exclusion localization sites were analyzed. In addition, transcription of the *Na^+/H^+ antiporter 1* (*NHX1*) gene was determined. Our findings can reveal the main mode of Na^+ exclusion in salt-tolerant buckwheat, and provide some theoretical basis for salt-tolerance in buckwheat.

Materials and methods

Plants, cultivation, and treatments: Salt-sensitive buckwheat (*Fagopyrum esculentum* Moench) cv. TQ-0808 and salt-tolerant cv. Chuanqiao No.1 were screened out and used as experimental materials (Ma *et al.* 2009). The

Submitted 26 December 2019, *last revision* 16 March 020, *accepted* 25 March 2020.
Abbreviations: NHX - Na^+/H^+ antiporter; RT-qPCR - quantitative real-time polymerase chain reaction; SOS - salt overly sensitive; TEM - transmission electron microscopy.
Acknowledgements: This work was supported by the National Natural Science Foundation of China (grant number 31371552). The first two authors contributed equally to this work.
* Corresponding author; e-mail: hbyang@qau.edu.cn

seeds were sterilized in 1 g dm^{-3} potassium permanganate for 10 min, and incubated in distilled water for 5 h, then germinated in incubator at 26 °C. After germination, the seedlings were moved to plastic pots with a Hoagland nutrient solution (pH 6.5 - 7.0) and cultivated under natural irradiance, day/night temperatures of 26/16 °C, and a relative humidity about 60 %. At the stage of 2 leaves and one central leaf, seedlings were treated with Hoagland nutrient solution containing 0 (control), 50, or 100 mM NaCl for 3 d, and the roots and stem bases were harvested for subsequent experiments. Each treatment was replicated 3 times.

Preparation and analysis by transmission electron microscopy (TEM): According to previous method (Lu *et al.* 1995), the materials for the TEM were washed with physiological brine, cut into small pieces about 1 mm^2, fixed at 4 °C in fixative [5 % $KSb(OH)_6$ (m/v), 2 % osmium tetroxide (m/v), pH 7.4] for 2 h, embedded with Spurr resin after cleaning, dehydration, and infiltration, polymerized at 65 °C for 24 h, cut into ultra-thin slices about 50 nm, and sprayed with a layer of carbon on the slice surface. Based on previous method (Guo *et al.* 1995), the samples were first photographed using TEM (*Hitachi H-7650*, Tokyo, Japan), and then used to analyze the Na^+ distribution with energy dispersive spectrometer (*EDAM II*, *EDAX*, Mahwah, NJ, USA), the measuring time was 200 s, the inclination was 0° , the extraction angle was 35°, the high voltage was 80 kV, the probe diameter was equal to or less than 1 μm, and each part of measurement was optionally chose for analysis at least 3 sites. The relative content of ion of the site was showed with "peak/back" value of ion.

Analysis of transcription: The quantitative real-time polymerase chain reaction (RT-qPCR) was employed. The stem bases of buckwheat seedlings were harvested for RNA isolation at the stage of two leaves and one central leaf. Total RNA was isolated using the *TRIzol* reagent (*Invitrogen*, Carlsbad, CA, USA), and contaminating genomic DNA was removed by RNase-free DNase I. The first cDNA strand was synthesized using *Superscript II* reverse transcriptase (*Invitrogen*). The RT-qPCR was performed using *SYBR Green I* master mix with the real-time PCR detection system (*Bio-Rad*, Hercules, USA) according to previous study (Ren *et al.* 2010). Gene-specific primer sequences were as follows: NHX1-F 5'-CGTTGCTAGGACGCAATGTTCCA-3' and NHX1-R 5'-ACAGTCCACGTCGGATGCCTTAT-3', SOS1-F 5'-CCTTACACCGTAGCTTT GCTC-3' and SOS1-R 5'-CCGGAAGAAACACAGCCAACA-3', *ACTIN* gene was used as a reference, its primer pairs were: ACTIN-F 5'-GCTGGATTTGCTGGAGATGATGC-3' and ACTIN-R 5'-CTTCTCCATGTCATCCCAGTTGCT-3'. The RT-qPCRs were repeated three times.

Statistical analysis: The data were subjected to Student's *t*-test (Wang *et al.* 2010) using *Microsoft Excel 2007* and *Sigma Plot 10.0.*

Results

As shown in Fig. 1*A*,*B*, the control buckwheat plants accumulated small amount of Na^+ in root cells, indicating that under normal physiological conditions, the absorbed Na^+ of buckwheat roots was generally used to maintain the normal function of cells. Under 50 mM NaCl, most Na^+ was accumulated in vacuole and less Na^+ was distributed in cell wall and cytoplasm of both buckwheat cultivars (Fig. 1*C*,*D*). Under 100 mM NaCl, more Na^+ was detected in buckwheat roots and similarly as at 50 mM treatment, Na^+ was mostly accumulated in vacuole (Fig. 1*E*,*F*). The amount of Na^+ accumulated in the vacuole of 'Chuanqiao No.1' was higher than that of 'TQ-0808' under 50 or 100 mM NaCl (Fig. 1*C-F*).

Energy dispersive spectrometer analysis of Na^+ in buckwheat root cells showed that in order to maintain normal physiological process, buckwheat absorbed and in control plants there were a certain amount of Na^+ in the cell wall or free space, cytoplasm, and vacuoles (Table 1). Under 50 mM NaCl, a lot of Na^+ was transported to the roots, and it tend to be accumulated mainly in vacuole (Table 1), which is benefit for normal physiological process of cell. With the increase of NaCl concentration (100 mM NaCl), more Na^+ was transported to the roots, but it still tended to entry the vacuole (Table 1). Under 50 mM NaCl, amount of Na^+ accumulated in cell wall or free space of 'Chuanqiao No.1' roots was 1.53-folds higher than in 'TQ-0808', and that in vacuoles of 'Chuanqiao No.1' roots it was 1.27-folds higher than in 'TQ-0808' (Table 1). On the contrary the Na^+ accumulation in cytoplasm of 'Chuanqiao No.1' roots was only 0.78-folds of that in cytoplasm of 'TQ-0808' (Table 1). Under 100 mM NaCl, the distribution of Na^+ in both buckwheat cultivars was similar as under 50 mM NaCl, and Na^+ accumulation in cell wall or free space of roots in 'Chuanqiao No.1' was 1.35-folds of that in 'TQ-0808', in vacuole was 1.41-folds, and in cytoplasm was 0.69-folds (Table 1). These results indicated that 'Chuanqiao No.1' could more effectively extruded Na^+ out of cell cytoplasm and compartmentalized Na^+ into the vacuole compared to 'TQ-0808'.

Under non-stressed conditions, the accumulation Na^+ in stem base cells was higher than that in roots (Figs. 1*A*,*B* and 2*A*,*B*). Like in root cells, Na^+ tended to be accumulated in vacuole of stem base cells of 'Chuanqiao No.1' (Fig. 2*B*), implying that stem base of salt-tolerant buckwheat was an important Na^+ exclusion tissue. Under 50 and 100 mM NaCl, higher amount of Na^+ was found in both cultivars, and the Na^+ accumulation in vacuole of 'Chuanqiao No.1' was higher than that in 'TQ-0808' (Fig. 2*C-F*). These results showed why 'Chuanqiao No.1' was more salt-tolerant than 'TQ-0808'.

Similarly as in root cells, the stem base cells of the control buckwheat accumulated small amount of Na^+ (Table 2). Under salt stress, Na^+ was accumulated mainly in vacuole, the following was the cell wall or free space, and the least amount was in cytoplasm (Table 2). Under 50 mM NaCl, amount of Na^+ accumulated in cell wall or free space of 'Chuanqiao No.1' stem base was 1.31-folds

Table 1. Distribution of Na^+ in root cells in buckwheat under NaCl stress (peak/back). Means ± SDs, $n = 3$, *, ** - statistically significant differences at $P < 0.05$ and $P < 0.01$, respectively.

NaCl [mM]	Cultivars	Cell wall and free space	Cytoplasm	Vacuole
0	TQ-0808	0.13 ± 0.02	0.06 ± 0.01	0.17 ± 0.03
	Chuanqiao No.1	0.20 ± 0.03	0.05 ± 0.01	0.24 ± 0.04
50	TQ-0808	0.17 ± 0.03	0.23 ± 0.03**	0.45 ± 0.06**
	Chuanqiao No.1	0.26 ± 0.03*	0.18 ± 0.03**	0.57 ± 0.07**
	Chuanqiao No.1/TQ-0808	1.53	0.78	1.27
100	TQ-0808	0.43 ± 0.05**	0.39 ± 0.05**	0.61 ± 0.07**
	Chuanqiao No.1	0.58 ± 0.07**	0.27 ± 0.04**	0.86 ± 0.10**
	Chuanqiao No.1/TQ-0808	1.35	0.69	1.41

of that in 'TQ-0808', and in vacuole of 'Chuanqiao No.1' stem base it was 1.28-folds higher than that in 'TQ-0808' (Table 2). In contrast, the Na^+ accumulation in cytoplasm of 'Chuanqiao No.1' stem base was 0.59-folds of that in 'TQ-0808' (Table 2). Under 100 mM NaCl, the distribution of Na^+ in both buckwheat stem base was similar to that under 50 mM NaCl, and Na^+ accumulation in cell wall or free space of stem base in 'Chuanqiao No.1' was 1.24-folds higher than in 'TQ-0808' and in vacuole and cytoplasm it was 1.38- and 0.51-folds higher, respectively (Table 2). These results indicating that 'Chuanqiao No.1' could effectively compartmentalize Na^+ into the vacuole or extrude Na^+ out of cell compared with 'TQ-0808'. In addition, we could find that under 50 or 100 mM NaCl, 'Chuanqiao No.1' could accumulate more Na^+ than 'TQ-0808', indicating that 'Chuanqiao No.1' could effective retain the Na^+ at stem base, thereby restricting its transport to the shoot.

As 'Chuanqiao No.1' could more effectively compartmentalize Na^+ into the vacuole compared with 'TQ-0808', we compared the *NHX1* and *SOS1* expression patterns in both buckwheat cultivars. As shown in Fig. 3, the transcription of *NHX1* and *salt overly sensitive 1* (*SOS1*) in 'Chuanqiao No.1' was significantly higher than in 'TQ-0808' in both roots and in stem base, which was consistend with the difference in the ability of Na^+ compartmentalization. These data demonstrated that the molecular mechanism of salt tolerance in 'Chuanqiao No.1' was due to its high *NHX1* and *SOS1* transcription.

Discussion

It has been reported that more than 95 % of Na^+ absorbed by salt-treated *Atriplex centralasiatica* plants accumulated in shoots, especially in leaves (Qiu *et al.* 2014), while in

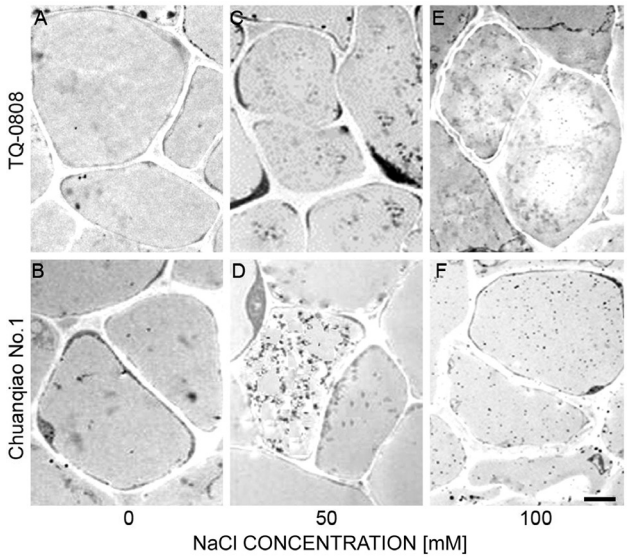

Fig. 1. Sodium ion localization in buckwheat root cells: *A* - cv. TQ-0808 control plants, *B* - cv. Chuanqiao No.1 control plants, *C* - 50 mM NaCl stressed 'TQ-0808', *D* - 50 mM NaCl stressed 'Chuanqiao No.1', *E* - 100 mM NaCl stressed 'TQ-0808', *F* - 100 mM NaCl stressed 'Chuanqiao No.1'. The *scale bar* is 5 μm.

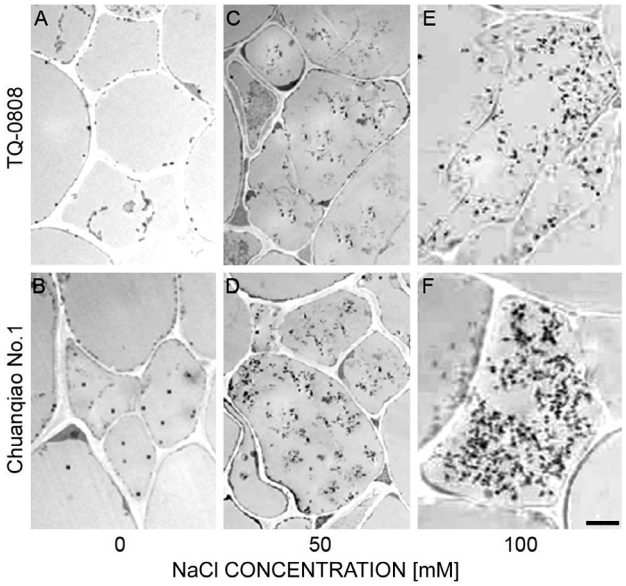

Fig. 2. Sodium ion localization in buckwheat stem base cells: *A* - cv. TQ-0808 control plants, *B* - cv. Chuanqiao No.1 control plants, *C* - 50 mM NaCl stressed 'TQ-0808', *D* - 50 mM NaCl stressed 'Chuanqiao No.1', *E* - 100 mM NaCl stressed 'TQ-0808', *F* - 100 mM NaCl stressed 'Chuanqiao No.1'. The *scale bar* is 5 μm.

Table 2. Distribution of Na^+ of stem base cells in buckwheat under NaCl stress (peak/back). Means ± SDs, n = 3, *, ** - statistically significant differences at $P < 0.05$ and $P < 0.01$, respectively.

NaCl [mM]	Cultivars	Cell wall and free space	Cytoplasm	Vacuole
0	TQ-0808	0.22 ± 0.03	0.12 ± 0.02	0.21 ± 0.02
	Chuanqiao No.1	0.23 ± 0.03	0.08 ± 0.01	0.25 ± 0.03
50	TQ-0808	0.35 ± 0.05*	0.27 ± 0.04**	0.53 ± 0.06**
	Chuanqiao No.1	0.46 ± 0.06**	0.16 ± 0.03*	0.68 ± 0.08**
	Chuanqiao No.1/TQ-0808	1.31	0.59	1.28
100	TQ-0808	0.50 ± 0.07**	0.43 ± 0.06**	0.71 ± 0.09**
	Chuanqiao No.1	0.67 ± 0.08**	0.22 ± 0.04**	0.98 ± 0.12**
	Chuanqiao No.1/TQ-0808	1.24	0.51	1.38

buckwheat, the roots and stem base were the main places of Na^+ localization (Ma *et al.* 2011). In this article, cortex of roots and parenchyma surrounding vascular bundle of stem base in buckwheat accumulated much more Na^+ than other parts. It indicated that buckwheat achieved the purpose of Na^+ exclusion from shoots by Na^+ accumulation in roots or stem bases especially in salt-tolerant buckwheat 'Chuanqiao No.1'. Therefore, the stem base of salt-tolerant buckwheat cultivar probably played an important role in restricting Na^+ transport to shoot.

Under salt stress, Na^+ extrusion from cytoplasm and compartmentalization in vacuole of plant cells was an important way to maintain low content of Na^+ in cytoplasm (Blumwald *et al.* 2000, Zhang and Blumwald 2001), and previous study proved that Na^+ compartmentalization had decisive role in salt tolerance of plants (Apse *et al.* 1999). Wang *et al.* (2019) found that Na^+ compartmentalization in vacuole of leaves was the major salt-adaptation strategy in Chinese cabbage. This study showed that Na^+ accumulation in roots and stem base played an important role in whole Na^+ exclusion of buckwheat. Under NaCl stress, Na^+ accumulation in cell wall or free space and vacuoles was significantly higher in salt tolerant than that in salt-sensitive cultivar. It indicated that Na^+ compartmentalization capability of salt-tolerant cultivar was significantly greater than that of salt-sensitive one, so that the Na^+ content of cytoplasm in roots or stem bases of salt-tolerant cultivar was significantly lower than that in salt-sensitive one. The higher *NHX1* and *SOS1* expressions in roots and stem bases in salt-tolerant cultivar provided related molecular evidence (Fig. 3).

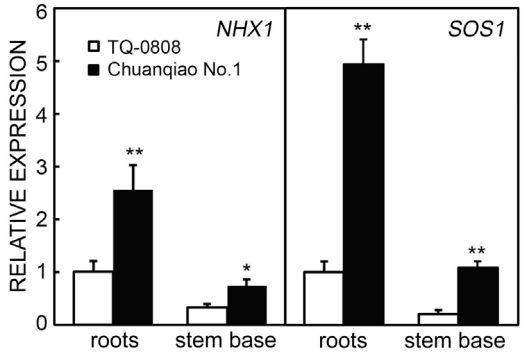

Fig. 3. *Na^+/H^+ antiporter 1* (*NHX1*) and *salt overly sensitive 1* (*SOS1*) transcriptions in the roots and stem bases of cvs. TQ-0808 and Chuanqiao No. 1. Means ± SDs, n = 3. Significant differences by Student's t-test (* - $P < 0.05$; ** - $P < 0.01$).

In conclusion, our results indicated that the capabilities of Na^+ extrusion from cytoplasm and Na^+ compartmentalization into vacuoles were obviously higher in salt-tolerant than in salt-sensitive buckwheat cultivars, and this capabilities could effectively restricted Na^+ transport to the shoot.

References

Apse, M.P., Aharon, G.S., Snedden, W.A., Blumwald, E.: Salt tolerance conferred by overexpression of a vacuolar Na^+/H^+ antiport in *Arabidopsis*. - Science **285**: 1256-1258, 1999.

Blumwald, E., Aharon, G.S., Apse, M.P.: Sodium transport in plant cells. - Biochim. biophys. Acta **1465**: 140-151, 2000.

Guo, N., Yang, Y., Sun, Y.X., Yu, M., Wang, B.Z., Zhang, D.T., Li, Y.G., Chen, D.H.: Ultrastructural observation on apoptosis of hybridoma cells induced by retroviral infection. - Chin. J. Phys. Med. **17**: 132-134, 1995.

Lu, J., Li, J., Hu, A., Li, X.: ESM observation of secretory salt structure in *Limonium bicolor* leaf. - Chin. J. appl. Ecol. **6**: 355-358, 1995.

Ma, D.Y., Li, F.L., Zhu, J.F., Zhan, W.Y., Yang, H.B.: Comparison of salt tolerance among six buckwheat varieties. - Mod. Agr. Sci. Technol. **3**: 157-158, 2009.

Ma, D.Y., Zhan, W.Y., Yang, H.B., Yi, Y.J.: Study on main Na^+ exclusion localization and Na^+/H^+ antiport activity of buckwheat. - Sci. agr. sin. **44**: 185-191, 2011.

Matsushita, N., Matoh, T.: Characterization of Na^+ exclusion mechanisms of salt-tolerant reed plants in comparison with salt-sensitive rice plants. - Physiol. Plant. **83**: 170-176, 1991.

Nie, L.L., Zhang, Y., Liu, Z.Q.: Mechanism of salt tolerance in plant. - Tianjin Agr. Sci. **14**: 6-9, 2008.

Qiu, N.W., Zhou, F., Wang, Y., Peng, X.Y., Hua, C.: The strategy of Na^+ compartmentation and growth of *Atriplex centralasiatica* in adaptation to saline environments. - Russ. J. Plant Physiol. **61**: 238-245, 2014.

Ren, X., Chen, Z., Liu, Y., Zhang, H., Zhang, M., Liu, Q., Hong, X., Zhu, J.K., Gong, Z.: Abo3, a wrky transcription factor, mediates plant responses to abscisic acid and drought tolerance in *Arabidopsis*. - Plant J. **63**: 417-429, 2010.

Schubert, S., Läuchli, A.: Sodium exclusion mechanisms at the root surface of two maize cultivars. - Plant Soil **123**: 205-209, 1990.

Wang, H.Y., Li, Y.: Research status and applied prospect of buckewheat. - Food Sci. **25**: 388-391, 2004.

Wang, J., Qiu, N., Wang, P., Zhang, W., Yang, X., Chen, M., Wang, B., Sun, J.: Na^+ compartmentation strategy of Chinese cabbage in response to salt stress. - Plant Physiol. Biochem. **140**: 151-157, 2019.

Wang, X.A., Yang, H.B., Qiu, N.W.: Application of paired-sample design in plant physiology experiment. - Plant Physiol. Commun. **46**: 161-164, 2010.

Wei, Y., Zhang, G., Sietz, W.: Studies on the quality properties of autotetraploid buckwheat kernel. - Sci. agr. sin. **28**(Suppl.): 34-40, 1995.

Yang, H., Han, Z., Xu, X.: Studies on Na^+ exclusion mechanism of three *maius* seedlings. - Acta hort. sin. **31**: 143-148, 2004.

Yang, H.B., Ding, S.H., Qiu, N.W., Wang, B.S., Cui, D.Y.: Na^+ exclusion by roots and root-stem junctions of wheat cultivars with different salt tolerance. - Acta photophysiol. sin. **272**: 179-185, 2001.

Zhan, W.Y., Zhou, G.K., Yang, H.B.: Na^+ localization and re-transportation of buckwheat seedlings. - Afr. J. agr. Res. **6**: 5835-5840, 2011.

Zhang, H.X., Blumwald, E.: Transgenic salt-tolerant tomato plants accumulate salt in foliage but not in fruit. - Natur. Biotechnol. **19**: 765-768, 2001.