The coordinated regulation of Na+ and K+ in *Hordeum brevisubulatum* responding to time of salt stress☆

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ARTICLE INFO

ABSTRACT

Hordeum brevisubulatum, called as wild barley, is a useful monocotyledonous halophyte for soil improvement in northern China. Although previously studied, its main salt tolerance mechanism remained controversial. The current work showed that shoot Na+ concentration was increased rapidly with stress time and significantly higher than in wheat during 0–168 h of 100 mM NaCl treatment. Similar results were also found under 25 and 50 mM NaCl treatments. Even K+ was increased from 0.01 to 50 mM in the cultural solution, no significant effect was found on tissue Na+ concentrations. Interestingly, shoot growth was improved, and stronger root activity was maintained in *H. brevisubulatum* compared with wheat after 7 days treatment of 100 mM NaCl. To investigate the long-term stress impact on tissue Na+, 100 mM NaCl was prolonged to 60 days. The maximum values of Na+ concentrations were observed at 7th in shoot and 14th day in roots, respectively, and then decreased gradually. Micro-electrode ion flux estimation was used and it was found that increasing Na+ efflux while maintaining K+ influx were the major strategies to reduce the Na+ concentration during long-term salt stress. Moreover, leaf Na+ secretions showed little contribution to the tissue Na+ decrease. Thereby, the physiological mechanism for *H. brevisubulatum* to survive from long-term salt stress was proposed that rapid Na+ accumulation occurred in the shoot to respond the initial salt shock, then Na+ efflux was triggered and K+ influx was activated to maintain a stable K+/Na+ ratio in tissues.

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1. Introduction

Salinity is a serious threat to crop yield as well as the environment protection [1]. Fortunately, halophytes have evolved various mechanisms to cope with soil salinity in long-term natural selection processes [2,3]. Most of the halophytes are dicotyledonous. However, the majority of the economically important crops are monocotyledonous [4,5]. Therefore, understanding of the salt-tolerance mechanism(s) of monocotyledonous halophytes, especially the wild relatives of cultivated cereals, will aid effective improvement of the salt tolerance of cereal crops [4–6].

Hordeum brevisubulatum (Trin.) Link, also known as wild barley, is a close relative of barley and wheat. It is well-known monocotyledonous halophytes and could be used as saline grass for soil improvement in north China [7]. The prime researches regarding the salt tolerance of *H. brevisubulatum* mainly focused on the description of the biological characteristics [8], along with the preliminary analysis of the physiological indexes during 1980–2000s [9]. Researches regarding the salt tolerance of *H. brevisubulatum* have gradually increased in recent years, but few have focused on its key physiological salt tolerance mechanism. Li et al. proposed that Na+ compartmentation in leaves and Na+ secretion from the leaf surface were the key adaptive mechanisms, but no obvious morphological evidence of secretory structures was found [10] except

for some called "salt hairs" [11], and no succulence was observed in the genus of *Hordeum*. Some other researchers suggested that restricting Na⁺ influx along with enhancing the osmotic adjustment by accumulating organic compounds can contribute to an overall enhancement of the salt tolerance in *H. brevisubulatum* [12,13]. On the other hand, construction of a cDNA library, the screening of expressed gene fragments induced by salt stress [14–17], analysis of the known salt tolerance related genes, such as *HbNHX1* [18], *DREB1* [19], *DREB2* [20], *rbcS* [21], *HbCDPK* [22] and *HbCIPK2* [7], and the subcellular localization of the protein for salt stress signal transduction gene, such as *CIPK* [23], *HbCBL1* and *HbCBL2* [24], were conducted by other researchers in order to find the main genes that control the salt tolerance of *H. brevisubulatum*. However, the main genes and physiological mechanisms for salt tolerance still remain unclear.

In current work, in order to find the key physiological salt tolerance mechanisms of *H. brevisubulatum*, firstly, the growth response indexes of shoot and root were determined after 7 days salt treatment of 100 mM NaCl. Interestingly, significantly higher shoot Na⁺ was accumulated rapidly in *H. brevisubulatum* than that in wheat. We also verified this under low concentrations (25, 50 mM). Meanwhile, treatment time was prolonged to 60 days to find the time point of peak values for Na⁺ and K⁺ concentration in tissues and their trends with the stress time prolonging. Surprisingly, Na⁺ was declined obviously during long-term stress. So, to assess the contribution of Na⁺ and K⁺ fluxes to the decline of tissue Na⁺ concentration, a micro-electrode ion flux estimation technique (MIFE) for net Na⁺ and K⁺ fluxes was used. Moreover, K⁺ concentration were expanded from 0.01 to 50 mM in medium to confirm whether high Na⁺ accumulation in shoot was caused by K⁺ deficiency in medium. Na⁺ content on the leaves surface was also measured to clarify the contribution of salt secretion to the salt tolerance of the plant species. This work would be also helpful to answer whether various mechanisms found by various researches to date are adopted during different growth stages in *H. brevisubulatum*.

2. Materials and methods

2.1. Plant materials and growth conditions

H. brevisubulatum seeds were collected from saline-alkaline wetlands (E100°06′, N 39° 11′) in northwestern China. Then, *H. brevisubulatum* and wheat (*Triticum aestivum* L. cv. "Longchun 26") seeds were germinated on bibulous paper saturated with sterile water in rectangular dishes (15 cm × 8 cm × 5 cm) in the dark at 25 °C. The germination period lasted 7 days for the *H. brevisubulatum* and 3 days for the wheat. After the leaf emergence occurred, seedlings were cultured with a modified Hoagland's nutrient solution (5 mM KNO₃, 1 mM NH₄H₂PO₄, 0.5 mM Ca(NO₃)₂, 0.5 mM MgSO₄, 60 μM Fe-Citrate, 92 μM H₃BO₃, 18 μM MnCl₂·4H₂O, 1.6 μM ZnSO₄·7H₂O, 0.6 μM CuSO₄·5H₂O and 0.7 μM (NH₄)₆Mo₇O₂₄·4H₂O) and the pH values were regulated as 6.0. Once the second leaf appeared, seedlings were transferred into black-painted containers with the same solution. Seedlings were grown in an environment controlled chamber at 25 °C in the day and 18 °C at night. There was a photon flux density of 2300 μmol m⁻² s⁻¹ in the day with a photo period of 16/8 h for the day/night cycle and a relative humidity from 65–75%. The solution was renewed every three days to avoid ion concentration imbalance.

For the pre-experiment, the plasma membrane permeability of the roots was determined under 0–300 mM NaCl with two, three and four-leaf-old plants in order to use the appropriate NaCl concentrations and growth stages (Supplementary Fig. S1). Then, four-leaf-old plants and 0–100 mM NaCl concentrations of

were used for the following experiments. Once the plants achieved four leaves, they were used for the salt treatment experiments. NaCl concentrations were increased by 25 mM/12 h in advance to achieve the final concentrations for treatments. The solutions were aerated and renewed every day to avoid oxygen and ion alterations.

2.2. Plant growth measurements

The plants of *H. brevisubulatum* and wheat were cultivated with a modified Hoagland's nutrient solution containing 25, 50, and 100 mM NaCl for 7 days. Then, the plants were harvested, gently blotted, separated into shoots and roots, weighed for fresh weights immediately and oven-dried at 80 °C for 3 d to obtain constant dry weights. Then, the water content and root/shoot ratio was calculated.

At the same time, root morphological parameters were determined using a root automatism scan apparatus (Perfection V700 Photo, Seiko Epson Corp, Japan) equipped with WinRHIZO software (Regent Instruments Co). The specific procedures were described in the references [25]. In each replicate, roots were placed in a transparent plastic tray filled with distilled water, and placed on the scan apparatus. Image recordings were performed at a resolution of 800 dpi, and saved as a tagged image file (TIF) format. The root phenotype traits, including the total root length, volume, surface area and number of root tips, were assayed using WinRHIZO software. The root-absorbing area was determined by a methylene-blue colorimetric method as described by Zou et al. [26]. The percentages of active absorption area and specific surface area were calculated according to Wang et al. [25].

2.3. Various terms of salt stress treatments

The plants of *H. brevisubulatum* and wheat were treated with a modified Hoagland nutrient solution supplemented with 25, 50 and 100 mM NaCl. Then, they were harvested for Na⁺ and K⁺ ion concentration measurements at 0, 6, 12, 24, 48, 96, and 168 h after salt stress for the short-term experiment and 7, 14, 28, and 60 days for the long-term experiment, respectively. The measurements of Na⁺ and K⁺ contents were carried out as described by Wang et al. [4]. Proline content in the tissues was tested according to Zhao [13].

2.4. Net Na⁺ and K⁺ fluxes measurements by MIFE

The net Na⁺ and K⁺ fluxes of *H. brevisubulatum* plants subjected to 100 mM NaCl were measured using a MIFE technique (BIO-IM, Younger USA LLC, Amherst, MA 01002, USA) as previously described [27–29] at Xuyue Science and Technology Co., Ltd, Beijing, China. In principal, prior to the flux measurement, the microelectrode was calibrated with different concentrations of the Na⁺ (0.3 mM, 0.9 mM and 3 mM), and K⁺ (0.1 mM, 0.5 mM and 5 mM) buffers. Only electrodes with a Nernstian slope >50 mV/decade were used in this study. The roots (with shoots retained) of the *H. brevisubulatum* were then washed gently with measuring solution and incubated in a petri dish containing a 10 ml of measuring solution (0.1 mM KCl, 0.1 mM CaC1₂, 0.1 mM MgC1₂, 0.5 mM NaCl, 0.2 mM Na₂SO₄, 0.3 mM MES, pH 6.0, adjusted with Tris) to equilibrate for 15 min. The net Na⁺ flux measurements commenced from the root tip and were repeated along the root at various positions from the root tip in order to determine the optimal position (the zone that corresponded to the site where the Caparian bands developed, and the zone of the lateral root's initial development). The steady-state of ion fluxes were then recorded until the values variation amplitude was relatively stable (approximately 400 s). The micro-electrode oscillated with an excursion of 30 μm, and completed an entire cycle in 5.36 s. The net ion fluxes (pmol cm⁻² s⁻¹) were calculated using Mage Flux software developed by Xuyue (http://xuyue.net/

mageflux). In order to eliminate the error of free diffusion during the testing caused by the differences of ion concentrations between the plants and test solutions during MIFE measuring processes, flux values of the control were considered as the reference values.

2.5. Various K+ level treatments

KNO$_3$ (5 mM) in Hoagland's nutrient solution was replaced by 0.01 mM KNO$_3$, 5 mM KNO$_3$, 5 mM KNO$_3$ + 15 mM KCl, and 5 mM KNO$_3$ + 45 mM KCl to achieved the final K$^+$ concentrations of 0.01, 5, 20 and 50 mM, respectively, while the NO$_3^-$ concentration was retained, under 100 mM NaCl for 14 days. 0.01 mM replaced 0 mM there to avoid nutrition deficiency. Then, the plants were harvested for Na$^+$ and K$^+$ ion concentration measurements. The selective absorption (SA) and selective transport (ST) values were calculated according to Wang et al. [30].

2.6. Salt secretion analysis

The salt secretion from the leaves was analyzed according to Wang et al. [4]. Briefly, four-leaf-old plants of *H. brevisubulatum* were cultured in hydroponic tanks and treated with 100 mM NaCl for 60 days; while control was irrigated in the same modified Hoagland solution without NaCl. Then, the leaves were carefully separated from the shoot bases, and rinsed in 30 ml of deionized water for 1.5 min, holding the incision above the water to avoid any Na$^+$ loss from the cut end. At this point, the leaves were removed from the washing water, blotted and weighted. The roots with the remaining shoots were harvested, and after washing off any surface soil with tap water, they were immediately blotted and weighed. The Na$^+$ and K$^+$ contents in the deionized water and the entire plants were measured as described below.

2.7. Statistical analysis

The results of the growth, ion concentration and net Na$^+$ and K$^+$ fluxes of the plants were presented as a means with standard deviations ($n = 6$). Statistical analyses, one-way analysis of variance (ANOVA), and Duncan's multiple range tests were performed using statistical software at $P < 0.05$ (Ver.16.0, SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Shoot growth was improved and stronger root activity was maintained in H. brevisubulatum compared with wheat during short-term salt stress

The growth responses in both plant species were investigated. Interestingly, it was found that fresh weight increased significantly in the shoots of *H. brevisubulatum* in 50 and 100 mM NaCl. However, no significant increases were observed in wheat (Fig. 1). The dry weights showed the similar results (Supplementary Fig. S2).

Total root length, root volume, root surface area, the numbers of root tip and percentage of active absorption area of wheat were significantly reduced by 7 day treatment of 100 mM NaCl compared to the control (Table 1). However, no significant negative effect was found for *H. brevisubulatum*, furthermore, the numbers of root tips, the percentage of active absorption and specific surface areas were even enhanced by 100 mM NaCl (Table. 1).

3.2. Rapid Na+ uptake and higher Na+ levels were maintained in the shoots of H. brevisubulatum during short-term (within 168 h) salt stress

Shoot Na$^+$ concentration of *H. brevisubulatum* was increased gradually with prolonged time and over 53-fold after 168 h treat-

ment of 100 mM NaCl, and it was significantly higher (2.6–6.4 folds) than that of wheat during 6–168 h (Fig. 2a). Na$^+$ concentrations in roots were relatively stable in both species (Fig. 2d). However, *H. brevisubulatum* showed an obviously lower root Na$^+$ concentration than in shoot. K$^+$ concentrations were relatively stable in shoot of both species during 0–168 h. Root K$^+$ concentrations decreased gradually from 48 h, but more stable and 29–40% higher K$^+$ concentrations were maintained in *H. brevisubulatum* than in wheat during 96–168 h of treatments (Fig. 2b and e). These results led to a lower K$^+$/Na$^+$ ratio in shoot, but a higher K$^+$/Na$^+$ ratio in root of *H. brevisubulatum* compared with wheat (Fig. 2c and f). Interestingly, even in the absence of NaCl, both shoot and root Na$^+$ concentrations were significantly higher (approximately 3 times) in *H. brevisubulatum* than those in wheat (Fig. 2a and d), leading to a significantly lower K$^+$/Na$^+$ ratio in *H. brevisubulatum* (Fig. 2c and f).

To clarify whether the high Na$^+$ concentration accumulation occurred under mild salt treatment in *H. brevisubulatum*, 25 and 50 mM NaCl were used. Similar result was obtained that higher Na$^+$ concentration was accumulated in shoot of *H. brevisubulatum* than wheat (Supplementary Figs. S3 and S4).

3.3. Na+ concentration was reduced while K+ was retained in H. brevisubulatum during long-term (7–60 days) NaCl treatments

To further assess whether the high Na$^+$ accumulation in *H. brevisubulatum* persisted over long-term treatments, time-courses (from 0 to 60 days) of the tissue K$^+$ and Na$^+$ concentrations were analyzed (Fig. 3a,b,c). The results indicated that shoot Na$^+$ concentration increased rapidly when subjected to 100 mM NaCl and reached the maximum value (2.15 mmol gDW^{-1}) at 7th days, and then gradually reduced with the prolonged NaCl treatments (14–60 days). Although root Na$^+$ concentration increased not so dramatically compared with shoot during 0–7 days, it increased sharply from 7th day and reached the maximum value (2.43 mmol gDW^{-1}) at 14th days, then decreased gradually till 60th day. It was noticeable that, although shoot Na$^+$ concentration increased rapidly and higher than in root during initial salt shock (0–7 days), relatively lower Na$^+$ levels was maintained in shoot during the following long-term stress (14–60 days) (Fig. 3a).

Although K$^+$ concentration had a slight decline during the short-term (0–7 day) stress, a relatively stable level of K$^+$ was maintained during the following stress. Consequently, K$^+$/Na$^+$ ratio had a slight decrease during 0–168 h (Fig. 2c and f), and then increased gradually (Fig. 3c). Moreover, K$^+$ concentrations were higher in shoots than those in roots during all time-courses (Fig. 3b).

3.4. Na+ and K+ fluxes contribution to Na+ decline during long-term (7–60 days) salt stress in H. brevisubulatum

In order to clarify the contribution of net Na$^+$ and K$^+$ fluxes to Na$^+$ decline during long-term stress, a MIFE technique at anatomically distinct zones were used. The results showed that a rapid Na$^+$ efflux (140.5 pmol cm^{-2} s^{-1}) was recorded after 7 days of 100 mM NaCl treatment compared with control, and then increased to 295.4 pmol cm^{-2} s^{-1} after 60 days of treatment (Fig. 4). Although, K$^+$ efflux (267.2 pmol cm^{-2} s^{-1}) was observed after 7 days of NaCl treatment, an obvious K$^+$ influx (-190.4 pmol cm^{-2} s^{-1}) was record after 60 days of treatment (Fig. 5). This was consistent with the results of tissue ion concentrations (Fig. 3) where Na$^+$ concentration decreased obviously from 7th day and K$^+$ concentration decrease at initial salt stock (0–7 days) and increased gradually in the following stages, consequently a higher K$^+$/Na$^+$ was maintained for *H. brevisubulatum* to adapt to salt stress.
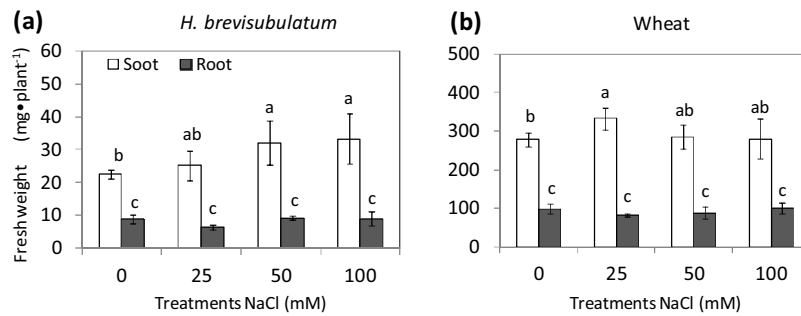
Fig. 1. Fresh weight of shoot (white columns) and root (black columns) in *H. brevisubulatum* (a) and wheat (b) under 0, 25, 50 and 100 mM NaCl (increased stepwise with 25 mM per 12 h) in modified Hoagland solution for 7 days. Ten plants for *H. brevisubulatum* and two plants for wheat were pooled in each replicate ($n=6$). Values are means ± SD and bars indicate SD.

Table 1

Root morphology and physiology indexes of *H. brevisubulatum* and wheat under control (0) and 100 mM NaCl (increased stepwise with 25 mM per 12 h) in modified Hoagland solution for 168 h. Ten plants for *H. brevisubulatum* and two plants for wheat were pooled in each replicate ($n=6$). Values are means ± SD and bars indicate SD.

Species	Treatments NaCl (mM)	Total root length (cm plant^{-1})	Root volume (mm^3 plant^{-1})	Root surface area (cm^2 plant^{-1})	Numbers of root tip (plant^{-1})	Percent of active absorption area (%)	Specific surface area (m^2 cm^{-3})
H. brevisubulatum	0	31.30 ± 2.54 a	9.98 ± 1.11 a	1.98 ± 0.22 a	140.68 ± 9.28 a	46.83 ± 0.26 b	1.52 ± 0.01b
	100	26.64 ± 3.09 a	8.85 ± 0.70 a	1.71 ± 0.11 a	89.28 ± 2.79 b	47.97 ± 0.23 a	1.56 ± 0.01 a
Wheat	0	159.68 ± 9.74 a	74.58 ± 5.15 a	12.19 ± 1.84 a	525.30 ± 35.24 a	48.54 ± 0.32 a	1.49 ± 0.01 b
	100	95.29 ± 12.67 b	63.25 ± 4.92 b	8.67 ± 0.72 b	286.80 ± 19.40 b	47.12 ± 0.20 b	1.54 ± 0.02a

Fig. 2. Na$^+$ (a), K$^+$ (b), K$^+$/Na$^+$ ratio (c) in shoot and Na$^+$ (d), K$^+$ (e), K$^+$/Na$^+$ ratio (f) in root of *H. brevisubulatum* (black columns) and wheat (white columns) during 0–168 h treatment of 100 mM NaCl (increased stepwise with 25 mM per 12 h) in modified Hoagland solution. Ten plants for *H. brevisubulatum* and two plants for wheat were pooled in each replicate ($n=6$). Values are means ± SD and bars indicate SD.

3.5. K$^+$ levels in medium had small effect on Na$^+$ accumulation in H. brevisubulatum

To confirm whether high Na$^+$ other than K$^+$ accumulation in *H. brevisubulatum* was caused by K$^+$ deficiency in medium, 5 mM K$^+$ in the solution was replaced by various concentrations of K$^+$ (0.01–50 mM) in 100 mM NaCl treatment. Shoot Na$^+$ concentration did not change significantly with 0.01, 5 and 20 mM K$^+$, but increased significantly by 37% with 50 mM K$^+$. No significant dif-
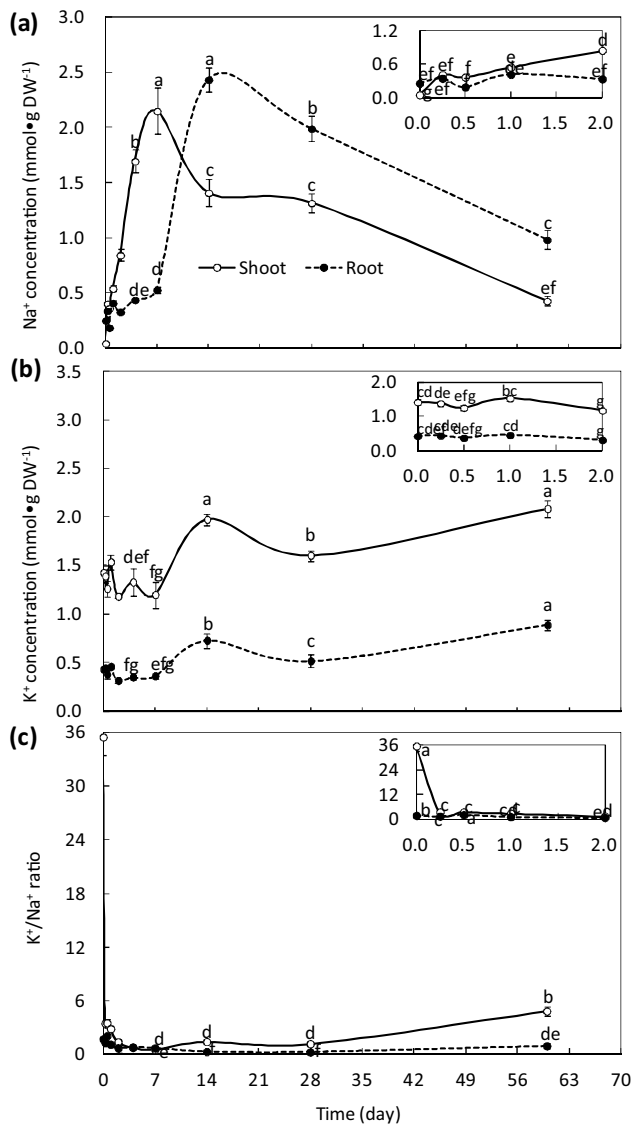
Fig. 3. Time course of Na$^+$ (a), K$^+$ (b), K$^+$/Na$^+$ ratio (c) in shoot (open circles) and root (closed circles) of *H. brevisubulatum* during 0–60 days under 100 mM NaCl (increased stepwise with 25 mM per 12 h) in modified Hoagland solution. The details of 0–2 days were showed in inserted figures. Ten plants for *H. brevisubulatum* were pooled in each replicate (*n* = 6). Values are means ± SD and bars indicate SD.
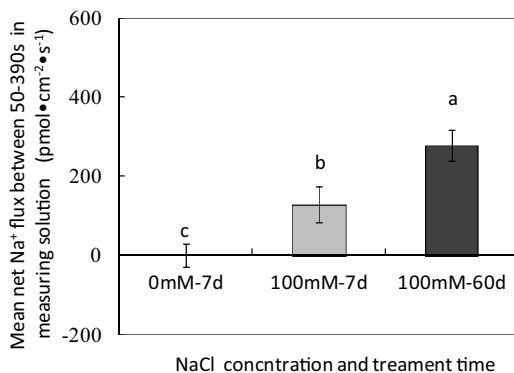

Fig. 4. Net Na$^+$ flux of *H. brevisubulatum* test by MIFE between 50 and 390 s in measuring solution after 0 and 100 mM NaCl treatment (increased stepwise with 25 mM per 12 h) in modified Hoagland solution for 7 d and 100 mM NaCl for 60 d. Roots (with shoots retained) were incubated in the measuring solution to equilibrate for 15 min in advance. Steady-state ion fluxes were then recorded until the values variation amplitude is relatively stable. Ten plants for *H. brevisubulatum* were pooled in each replicate (*n* = 6). Values are means ± SD and bars indicate SD.

Fig. 5. Net K$^+$ flux of *H. brevisubulatum* test by MIFE between 50 and 390 s in measuring solution after 0 and 100 mM NaCl treatment (increased stepwise with 25 mM per 12 h) in modified Hoagland solution for 7 d and 100 mM NaCl for 60 d. Roots (with shoots retained) were incubated in the measuring solution to equilibrate for 15 min ahead. Steady-state ion fluxes were then recorded until the values variation amplitude is relatively stable. Ten plants for *H. brevisubulatum* were pooled in each replicate (*n* = 6). Values are means ± SD and bars indicate SD.
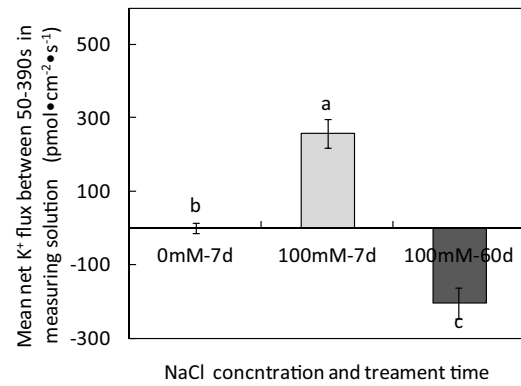
Table 2
Selective absorption (SA) and transport (ST) capacity for K$^+$ over Na$^+$ of *H. brevisubulatum* exposed to 100 mM NaCl supplied with 0.01, 5, 20 and 50 mM K$^+$ (NaCl increased stepwise with 25 mM per 12 h) in modified Hoagland solution for 14 d. Ten plants for *H. brevisubulatum* were pooled in each replicate (*n* = 6). Values are means ± SD and bars indicate SD. The values were calculated from Fig. 6. SA = (K$^+$/Na$^+$ in whole plant)/(K$^+$/Na$^+$ in medium); ST = (K$^+$/Na$^+$ in shoots)/(K$^+$/Na$^+$ in roots).

K$^+$ concentration (mM)	SA	ST
0.01	–	10.15 ± 1.03 a
5	119.01 ± 6.57 a	5.67 ± 0.54 b
20	33.19 ± 1.96 b	5.52 ± 0.35 b
50	12.58 ± 0.89 c	3.56 ± 0.38 c

ferences in root Na$^+$ concentrations were found among 5, 20 and 50 mM K$^+$ treatments, even significantly decrease occurred in these three K$^+$ levels compared with 0.01 mM K$^+$. Tissue K$^+$ concentration increased significantly with the increase of K$^+$ concentrations in medium, and reached the maximum values with 50 mM K$^+$ (Fig. 6b).

The selective absorption (SA) and selective transport (ST) values for K$^+$ over Na$^+$ were calculated and confirmed that SA values decreased sharply, while the ST values remain relatively stable with the addition of 5–50 mM K$^+$ (Table 2).

3.6. Loss of Na$^+$ content from the leaves

To assess the contribution of salt secretion from the leaves to the decline of Na$^+$ concentration in shoots during long-term salt stress, Na$^+$ and K$^+$ contents on leaves surface was determined under 100 mM NaCl for 60 days. Na$^+$ content washed from the leaves were not significantly different between 0 and 100 mM NaCl treatments accounting for only 2.52% of the entire plant Na$^+$ content with 100 mM NaCl (Fig. 7a). K$^+$ content washed from the leaves accounted only about 0.1% of the entire plant K$^+$ content with 0 and 100 mM NaCl.

4. Discussion

4.1. Rapid and higher Na$^+$ accumulation in shoot was a rapid response to short-term salt stress

Excessive Na$^+$ induces osmotic stress and cytosolic toxicity, resulting in growth inhibition for glycophytes, and Na$^+$ also acts as a competitor of K$^+$ with similar binding sites in major metabolic processes [31,32]. Therefore, limiting high Na$^+$ concentration accu-
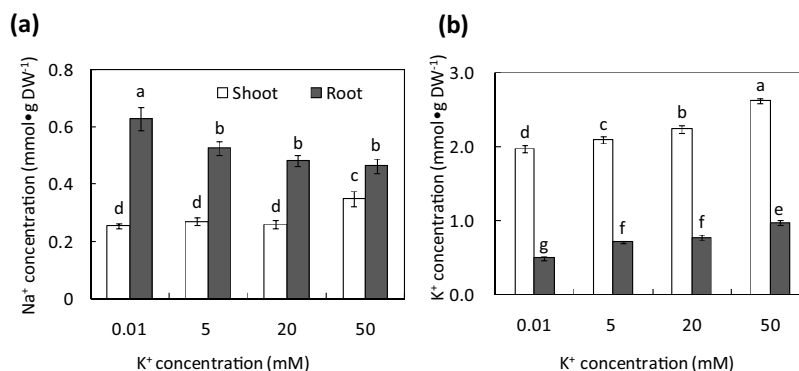
Fig. 6. The influence of K$^+$ level (0.01–50 mM) on Na$^+$ (a) and K$^+$ (b) concentration of *H. brevisubulatum* under 100 mM NaCl (increased stepwise with 25 mM per 12 h) in modified Hoagland solution for 14 d. Ten plants for *H. brevisubulatum* were pooled in each replicate (*n* = 6). Values are means ± SD and bars indicate SD.
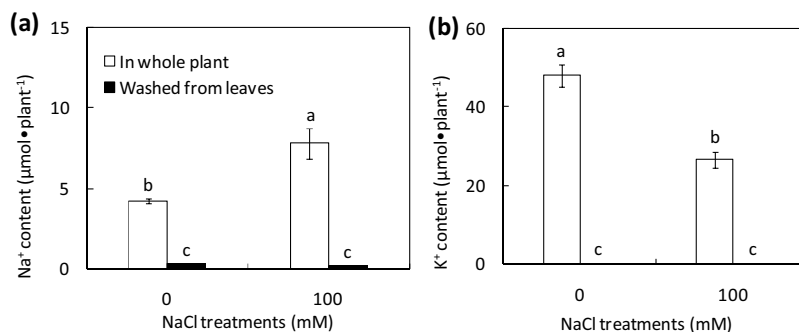

Fig. 7. Na$^+$ content loss from leaves of *H. brevisubulatum* under 0 and 100 mM NaCl (increased stepwise with 25 mM per 12 h) in modified Hoagland solution for 60 d. Ten plants for *H. brevisubulatum* were pooled in each replicate (*n* = 6). Values are means ± SD and bars indicate SD.

mulation as a major salt tolerance strategy is adopted by most monocotyledonous halophytes [3–5,33].

However, in present study, rapid and high Na$^+$ was accumulated in the shoots, other than in roots of *H. brevisubulatum*, even over 2-fold of wheat during short-term salt stress (Fig. 2a). Interestingly, although Na$^+$ levels were higher in *H. brevisubulatum*, growth rates of the shoots and root activity remained higher than in wheat (Fig. 1a, Table 1). Li et al. found that Na$^+$ could probably be compartmentalized into vacuole in *H. brevisubulatum* [10]. It was also found that Na$^+$ concentration in barley increased rapidly and sequestered into the leaves during the first several days [28], and tolerant barley genotypes accumulated significantly higher Na$^+$ in their leaves compared with sensitive ones [34]. Similar results were also found in *Thellungiella halophila* exposed to salt stress [35]. The most probable explanation is that the plants undergoing salt stress could send the amount of required Na$^+$ quickly to the shoot, as a 'cheap' osmoticum to achieve a rapid full osmotic adjustment [36], rather than simply restricting Na$^+$ influx at the beginning stage of salt stress. This consequently provides an additional driving force for water uptake by roots [37] and resumes growth of shoots (Fig. 1a). In contrast, it was found that glycophytes like wheat (Fig. 2a) and pea [38] could restrict xylem Na$^+$ loading during the initial few days of the salt stress treatments, but failed to prevent Na$^+$ elevation over longer term exposure to salinity.

An improved Na$^+$ sequestering ability in the leaf vacuoles of *H. brevisubulatum* could be achieved by tonoplast Na$^+$/H$^+$ exchanger, HbNHX1 [18,39]. In the present study, it is interesting to note that rapid and higher Na$^+$ accumulation was also found in the lower (25 and 50 mM) NaCl treatments (Supplementary Figs. S3 and S4), consistent with the result of RT-PCR identification with *HbNHX1* not induced by salt stress [18]. It was proposed that Na$^+$ transport under mild salinity was mediated by AtSOS1 (Salt Overly Sensitive 1), which is located across the plasma membrane of xylem parenchyma cells and could mediate Na$^+$ loading into the xylem, thereby controlling the long-distance Na$^+$ transport from roots to shoots [40,41]. Under higher saline conditions, AKT1 (*Arabidopsis* K$^+$ transporter) regulated Na$^+$ uptake in the roots [31] and its expression was not influenced by outside K$^+$ concentrations [39,42]. These finding were consistent with the results in current study that Na$^+$ concentrations in the roots were not influenced by outside K$^+$ levels from 5 to 50 mM (Fig. 6a). Our previous results in the salt-accumulating halophyte *Suaeda maritima* also supported that AKT type K$^+$ channels may be involved in Na$^+$ uptake in the roots under 150 mM NaCl [43].

4.2. Na$^+$ efflux was the major mechanism for H. brevisubulatum to maintain a low Na$^+$ accumulation during long-term salt stress

Although high Na$^+$ levels was rapidly observed in shoots of *H. brevisubulatum* (Fig. 2a), once this was achieved to the limitation of the vacuolar volume, it was better for the plants to reduce Na$^+$ loading rate, increase Na$^+$ retrieval from shoots to roots and exclude Na$^+$ to the soil [44,45]. In current study, Na$^+$ concentration in shoots reached its maximum values at 7th day of the treatments (Fig. 3a) and then declined gradually. Consistently, Na$^+$ concentration in roots increased obviously from 7th day and reached the maximum values at 14th day (Fig. 3a). It was suggested that AtHKT1;1 (High-affinity K$^+$ Transporter) located at the plasma membrane of xylem parenchyma cells could mediated Na$^+$ retrieval from the xylem to the surrounding parenchyma cells [46,47], and thereby regulated Na$^+$ transport from shoots to roots [48–50]. Consistently, *athkt1;1* mutant accumulated more Na$^+$ in shoot and less Na$^+$ in roots compared with the wild type plants [49,50], indicating that AtHKT1;1 could be a determinant to controlling Na$^+$ unloading from the xylem.

With large amount of Na^+ retrieval from shoot to root, Na^+ was efflux from roots to external environment (Fig. 4). Then, Na^+ concentrations in roots were consequently reduced from 14th day (Fig. 3a). A plasma membrane-bound Na^+/H^+ antiporter [39], encoded by *SOS1* at the epidermal cells of the root tips [40], is crucial in mediating Na^+ efflux from roots to the environment [51–53]. It was noted that, regardless of halophytes or glycophytes, unidirectional Na^+ efflux accounted for large amounts of total Na^+ influx under salinity conditions [4,54–56]. Na^+ concentrations both in shoots and roots (Fig. 3a) decreased with the increase of Na^+ efflux (Fig. 4), suggesting that Na^+ efflux from roots was the main contributor to reduced Na^+ concentration during the long-term salt stress in *H. brevisubulatum*. The similar result showed that a better ability of root cells to pump Na^+ from cytosol to external medium was also found in salt-tolerant varieties of barley [34], and accumulated approximately 30% less Na^+ than in salt-sensitive varieties after four weeks of salt stress [28].

4.3. Coordinated regulation of K^+ and Na^+ in H. brevisubulatum under salinity stress

Maintaining a high-cytosolic K^+/Na^+ ratio is one of the crucial and essential mechanisms of salt tolerance [31,34,48]. The coordinated regulation of K^+ and Na^+ (not simply maintaining a high K^+ and low Na^+) may play an important role for salt tolerance in *H. brevisubulatum*. As described above, Na^+ uptake in *H. brevisubulatum* increased rapidly and was higher than in wheat during the first 168 h of salt stress (Fig. 2a). This was probably due to the AKT1-type channels [57,58], the subfamily 1-type HKT transporters [59–61] on root epidermal cells and SOS1 on xylem parenchyma cells. They together promoted the uptake and long distance transport of Na^+ [44,45] into cell vacuoles of shoots to achieve a higher osmotic potential. Na^+ unloading into the xylem parenchyma cells possibly depolarizes their plasma membrane, which in turn could potentially activate the K^+ channels, such as SKOR (Stelar K^+ Outward Rectifiers), to load K^+ into xylem [45,62,63]. Therefore, K^+ concentrations increased during 7–14 days in both in shoot and root (Fig. 3b), following the rapid Na^+ uptake during the first 7 days (Fig. 3a). When Na^+ content achieved its limitation of vacuolar volume, the subfamily 1-type HKT transporters mediated the Na^+ retrieval from shoots to roots [44,45]. Then, Na^+ concentration declined in the shoots and increased in the roots during 7 to14 days of salt stress (Fig. 3a).

Once excessive Na^+ accumulated in the root cells (Fig. 3a), K^+ efflux from epidermis cells was triggered due to salt-induced membrane depolarization [64,65] (Fig. 5), probably via the GORK(Guard cell Outward Rectifying K^+ channel) [66]. This consequently led to a decrease in K^+ concentration in both shoots and roots during 14–28 days of treatment (Fig. 3b). Then, with the increase of Na^+ efflux in roots (Fig. 4), Na^+ concentration obviously decreased during 28–60 days (Fig. 3a) and K^+ influx recovered, thereby K^+ concentration and K^+/Na^+ ratio increased progressively (Fig. 3b and c). This was consistent with the MIFE results that K^+ maintained a higher efflux at 7th day, but recovered influx at 60th day under 100 mM NaCl treatments compared with control (Fig. 5).

Accordingly, by integrating all the findings as described above, a probable pattern for elucidating salt tolerance mechanism of *H. brevisubulatum* was proposed, in which the roles of K^+ and Na^+ coordinated regulations during short and long-term salt stress were clarified (Fig. 8). During the first stage (0–7 days; Fig. 2a), Na^+ was absorbed quickly into the epidermal cells of roots, probably via AKT1 [57,58] or HKT (the subfamily 1-type HKT transporters) [59,60], then was loaded directly into the xylem via SOS1 in the plasma membranes of XPCs (Xylem Parenchyma Cell) and delivered rapidly to the shoots motivated by transpiration stream [28,44,45]. Then, large amount of Na^+ was rapidly sequestered into leaf vac-
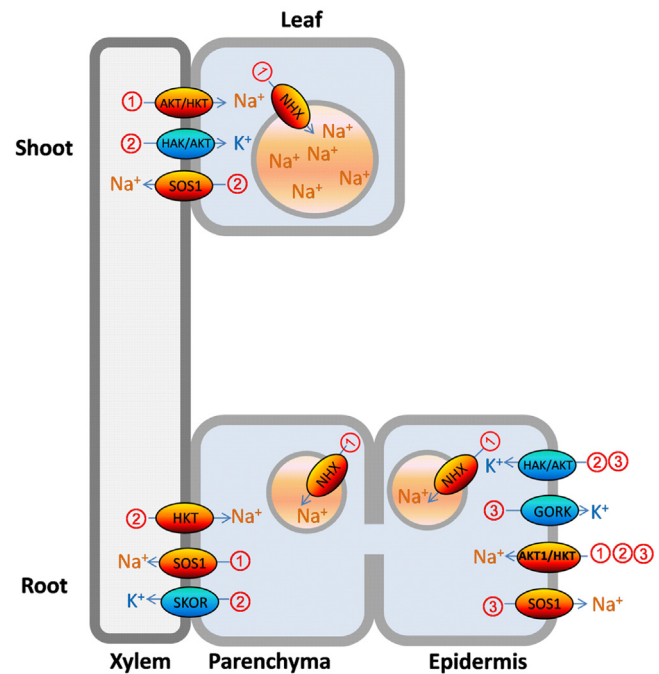

Fig. 8. A proposed schematic pattern for K^+ and Na^+ transporters/channels to coordinate regulation of salt tolerance of *H. brevisubulatum*. ① During the first stage (0–7 days), Na^+ was absorbed quickly into the epidermal cells of roots, probably via AKT1 or HKT, then was loaded directly into the xylem via SOS1 in the plasma membranes of XPCs and delivered rapidly to the shoots motivated by transpiration stream. Then, large amount of Na^+ was rapidly sequestered into leaf vacuoles via NHX, in order to achieve rapid full osmotic adjustment and avoid Na^+ damage. ② During the second stage, once Na^+ achieved its limitation in vacuole, HKT could then mediate Na^+ retrieval from xylem into XPCs to limit Na^+ accumulation in shoots, and relatively larger amount of Na^+ was accumulated in roots. Then in turn, this could activate K^+ channels, such as SKOR, to load the K^+ into xylem through XPCs, and finally promote the K^+ uptake into the plants possibly via HAK or other K^+ uptake transporters or channels. ③ During the third stage, once Na^+ was retrieved and excessively accumulated in root cells, Na^+ efflux possibly via SOS1 on root epidermal cells was triggered. At the same time, K^+ efflux via K^+ efflux channels such as GORK due to the salt-induced membrane depolarization from the cells was also triggered, leading to a decrease in K^+ concentrations in the plants. Then, with the increase of Na^+ efflux, Na^+ concentration decreased and K^+ concentration recovered gradually by its uptake, resulting in a higher K^+/Na^+, which consequently was sustained during long term of higher salt stress.

uoles via tonoplast Na^+/H^+ exchanger (NHX) [18,39], in order to achieve rapid full osmotic adjustment and avoid Na^+ damage [36]. During the second stage, once Na^+ achieved its limitation in vacuole, HKT could then mediate Na^+ retrieval from xylem into XPCs to limit Na^+ accumulation in shoots [44,45,47,67], and relatively larger amount of Na^+ was accumulated in roots (Fig. 3a, days 7–14).Then in turn, this could activate K^+ channels, such as SKOR, to load the K^+ into xylem [45,62,63] through XPCs, and finally promote the K^+ uptake into the plants [28,48,50] possibly via HAK or other K^+ uptake transporters or channels. During the third stage, once Na^+ was retrieved and excessively accumulated in root cells (Fig. 3a, days 7–14), Na^+ efflux possibly via SOS1 on root epidermal cells was triggered [40,52,67,68]. At the same time, K^+ efflux via K^+ efflux channels such as GORK due to the salt-induced membrane depolarization from the cells was also triggered [66], leading to a decrease in K^+ concentrations in the plants (Fig. 3b, days 14–28). Then, with the increase of Na^+ efflux (Fig. 4), Na^+ concentration decreased and K^+ concentration recovered gradually by its uptake, resulting in a higher K^+/Na^+ (Fig. 3a, b, c, and Fig. 5), which consequently was sustained during long term of higher salt stress.

4.4. Na⁺ loss from leaves makes a minor contribution to salt tolerance of H. brevisubulatum

Although high Na^+ level was maintained in shoots of *H. brevisubulatum*, Na^+ loss from leaf surface was extremely minor of Na^+ amount in the entire plant (2.6%, Fig. 7) compared with salt-secreting halophytes, such as *Spartina anglica* (60%), *Limonium vulgare* (33%), *Glaux maritime* (20%) and wild rice Porteresiacoarctata (over 50%) [69,70]. Moreover, although some "salt hairs" were observed on the leaves [11], no salt-secreting structures was observed in *H. brevisubulatum* [10], also no bicellular glands was observed in any other species of the Pooideae family [71], indicating that Na^+ secreting contributes little to salt tolerance of *H. brevisubulatum*.

5. Conclusion

This study provided evidence that rapid Na^+ influx occurred at seedling stages with initial salt shock and Na^+ efflux and K^+ influx was enhanced to maintain a K^+ and Na^+ balance at tilling stages during long-term salt stress in the monocotyledonous halophyte *H. brevisubulatum*. A probable regulation pattern was hypothesized to elucidate its K^+ and Na^+ co-ordinated mechanism against different terms of salt stress. Although this seems a smart strategy, it is too early to speculate that this mechanism could be adopted by other wild plant species.

Acknowlegements

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.plantsci.2016.08.009.

References

[1] J. Millar, J. Roots, Changes in Australian agriculture and land use: implications for future food security, Inter. J. Agr. Sust. 10 (2012) 25–39.
[2] M. Tester, R. Davenport, Na⁺ tolerance and Na⁺ transport in higher plants, Ann. Bot. 91 (2003) 503–527.
[3] T.J. Flowers, T.D. Colmer, Salinity tolerance in halophytes, New Phytol. 179 (2008) 945–963.
[4] C.M. Wang, J.L. Zhang, X.S. Liu, Z. Li, G.Q. Wu, J.Y. Cai, T.J. Flowers, S.M. Wang, *Puccinellia tenuiflora* maintains a low Na⁺ level under salinity by limiting unidirectional Na⁺ influx resulting in a high selectivity for K⁺ over Na⁺, Plant Cell Environ. 32 (2009) 486–496.
[5] Y.H. Peng, Y.F. Zhu, Y.Q. Mao, S.M. Wang, W.A. Su, Z.C. Tang, Alkali grass resists salt stress through high [K⁺] and an endodermis barrier to Na⁺, J. Exp. Bot. 55 (2004) 939–949.
[6] S.M. Wang, G.Q. Zhao, Y.S. Gao, Z.C. Tang, C.L. Zhang, *Puccinellia tenuiflora* exhibits stronger selectivity for K⁺ over Na⁺ than wheat, J. Plant Nutr. 27 (2005) 1841–1857.
[7] R.F. Li, J.W. Zhang, G.Y. Wu, H.Z. Wang, Y.J. Chen, J.H. Wei, HbCIPK2 a novel CBL-interacting protein kinase from halophyte *Hordeum brevisubulatum*, confers salt and osmotic stress tolerance, Plant Cell Environ. 35 (2012) 1582–1600.
[8] D.W. Jin, Z.S. Wang, The wild forage grass in Jilin province, wild barley, Jilin Agric. Sci. 2 (1981) 73–76 (in Chinese).
[9] P. Wang, J.X. Guo, Growth and physiological metabolic changes of *Hordum brevisublatum* under different abversites, J. Northeast Norm. Univ. 25 (1998) 120–123 (in Chinese with English Abstract).
[10] R.F. Li, X.Q. Wang, H.Z. Wang, J.H. Wei, Adaptive mechanisms of salt tolerance in *Hordeum brevisubulatum*, Sci. Agric. Sin. 39 (2006) 2459–2466 (in Chinese with English Abstract).
[11] R.F. Li, J.H. Wei, H.Z. Wang, Study of salt tolerance in barley under NaCl stress, Pratacultural Sci. Suppl. 7 (2004) 516–522 (in Chinese).
[12] X.Q. Wang, J.W. Zhang, J.H. Wei, H.Z. Wang, R.F. Li, Primary studies on physiological mechanisms of salt tolerance in *Hordeum brevisubulatum* under salt stress, Acta Agric. Boreali Sin. 22 (2007) 17–21 (in Chinese with English Abstract).
[13] Y. Zhao, Determination of Betaines and Proline in Plant Tissues Under Salt-Stress, Master Degree Thesis, CAAS, 2004 (in Chinese with English Abstract).
[14] X.Q. Qi, Cloning of Gene(s) Induced by Salt Stress & Analysis of Its Function in Wild Barley (*Hordeum Brevisubulatum* (Trink.) Link.), Master Degree Thesis, Chinese Academy of Agricultural Sciences, 2004 (in Chinese with English Abstract).
[15] Y.M. Lu, Y.F. Li, J.Y. Bai, M.F. Cao, H.W. Ma, C.M. Ruan, Construction of cDNA library of *Hordeum brevisubulatium* Link under salt stress, J. Jilin Agric. Univ. 25 (2003) 499–502 (in Chinese with English Abstract).
[16] X.Q. Wang, Screening and Cloning of Salt-inducible Gene in Shortsubulate Barley (*Hordeum Brevisubulatum* (Trink.) Link.), Master Degree Thesis, Capital Normal University, 2006 (in Chinese with English Abstract).
[17] L. Zhou, Difference in Gene Sequence Polymorphis & Gene Expression Under Salt Stress Between Wild Type Barley and Salt-tolerance Barley (*Hordeum Brevisubulatum* (Trink.) Link.), Master Degree Thesis, Jilin University, 2010 (in Chinese with English Abstract).
[18] S.Y. Lü, Y.X. Jing, S.H. Shen, H.Y. Zhao, L.Q. Ma, X.J. Zhou, Q. Ren, Y.F. Li, Antiporter gene from *Hordeum brevisubulatum* (Trin.) link and its overexpression in transgenic tobaccos, J. Integr. Plant Boil. 47 (2005) 343–349.
[19] C.Y. Wang, M.Z. Zhang, S. Wan, S.Y. Lu, L.Q. Ma, Y.F. Li, Cloning and characterization of HbDREB1 gene encoding a putative DRE-binging transcription factor in *Hordeum brevisubulatum*, J. Jilin Agric. Univ. 28 (2006) 516–520 (in Chinese with English Abstract).
[20] C.Y. Wang, M.Z. Zhang, S. Wan, Y.F. Li, Clone of HbDREB2 gene of *Hordeum brevisubulatum* and identification of its transgenic tobaccos, J. Jilin Agric. Univ. 29 (2007) 643–646 (in Chinese with English Abstract).
[21] H.Y. Yue, J.R. Yin, S.Q. Yan, Y.L. Feng, L.J. Zhang, J.Q. Guo, H.L. Li, X.M. Ding, J.L. Shen, Cloning and sequence analysis of *rbcS* gene of wild barley (*Hordeum brevisubulatum*) under salt stress, Agric. Sci. Technol. 11 (2010) 42–44.
[22] C.Y. Wang, M.Z. Zhang, S.Y. L, Y.F. Li, Molecular cloning and functional analysis of a novel calcium-dependent protein kinase gene HbCDPK from *Hordeum brevisubulatum*, J. Northwest A & F Univ. (Nat Sci. Ed.) 39 (2011) 111–119 (in Chinese with English Abstract).
[23] S.S. Li, G.S. Kan, J.H. Wei, R.F. Li, The subcellular localization of CIPK from *Hordeum brevisubulatum*, Liaoning Agric. Sci. 4 (2011) 1–5 (in Chinese with English Abstract).
[24] G.Y. Wu, G.J. Li, R.G. Wang, R.F. Li, J.H. Wei, Analyses for protein structure and subcellular localization of HbCBL1 and HbCBL2 from *Hordeum brevisubulatum*, Acta Agric. Boreali Sin. 26 (2011) 1–7 (in Chinese with English Abstract).
[25] C.M. Wang, The Function of Sodium on Stress Adaptation in Salt Excluding Plant *Puccinellia Tenuiflora* and Salt Accumulating Plant *Zygophyllum Xanthoxylum*, Master Degree Thesis, Lanzhou University, 2008 (in Chinese with English Abstract).
[26] Q. Zou, Experimental Guidance of Plant Physiology, China Agriculture Press, Beijing, 2000.
[27] J. Sun, S. Chen, S. Dai, R. Wang, N. Li, X. Shen, X. Zhou, C. Lu, X. Zheng, Z. Hu, Z. Zhang, J. Song, Y. Xu, NaCl-induced alternations of cellular and tissue ion fluxes in roots of salt-resistant and salt-sensitive poplar species, Plant Physiol. 149 (2009) 1141–1153.
[28] S. Shabala, S. Shabala, T.A. Cuin, J.Y. Pang, W. Percey, Z.H. Chen, S. Conn, C. Eing, L.H. Wegner, Xylem ionic relations and salinity tolerance in barley, Plant J. 61 (2010) 839–853.
[29] Q. Zhou, L. Wang, X. Cai, D. Wang, X. Hua, L. Qu, J. Lin, T. Chen, Net sodium fluxes change significantly at anatomically distinct root zones of rice (*Oryza sativa* L.) seedlings, J. Plant Physiol. 168 (2011) 1249–1255.
[30] S.M. Wang, G.Q. Zhao, Y.S. Gao, Z.C. Tang, C.L. Zhang, *Puccinellia tenuiflora* exhibits stronger selectivity for K⁺ over Na⁺ than wheat, J. Plant Nutr. 27 (2004) 1841–1857.
[31] F.J. Maathuis, A. Amtmann, K⁺ nutrition and Na⁺ toxicity: the basis of cellular K⁺/Na⁺ ratios, Ann. Bot. 84 (1999) 123–133.
[32] S. Shabala, T.A. Cuin, Potassium transport and plant salt tolerance, Physiol. Plant 133 (2008) 651–669.
[33] Q. Guo, L. Meng, P.C. Mao, X.X. Tian, Salt tolerance in two tall wheatgrass pecies is associated with selective capacity for K⁺ over Na⁺, Acta Physiol. Plant 37 (2015) 1–9.
[34] Z.H. Chen, I.I. Pottosin, T.A. Cuin, A.T. Fuglsang, M. Tester, D. Jha, I. Zepeda-Jazo, M.X. Zhou, M.G. Palmgren, I.A. Newman, S. Shabala, Root plasma membrane transporters controlling K⁺/Na⁺ homeostasis in salt stressed barley, Plant Physiol. 145 (2007) 1714–1725.
[35] F. Alemán, M. Nieves-Cordones, V. Martínez, F. Rubio, Potassium/sodium steady-state homeostasis in *hellungiella halophila* and *Arabidopsis thaliana* under long-term salinity conditions, Plant Sci. 176 (2009) 768–774.
[36] S. Shabala, Learning from halophytes: physiological basis and strategies to improve abiotic stress tolerance in crops, Ann. Bot. 112 (2013) 1209–1221.
[37] J.S. Boyer, Water transport, Annu. Rev. Plant Physiol. 36 (1985) 473–516.

[38] J. Bose, L. Shabala, I. Pottosin, F. Zeng, A.M. Velarde-buendía, A. Massart, C. Poschenrieder, Y. Hariadi, S. Shabala, Kinetics of xylem loading, membrane potential maintenance, and sensitivity of K^+-permeable channels to reactive oxygen species: physiological traits that differentiate salinity tolerance between pea and barley, Plant Cell Environ. 37 (2014) 589–600.

[39] E. Blumwald, G.S. Aharon, M.P. Apse, Sodium transport in plant cells, Biochim. Biophys. Acta 1465 (2000) 140–151.

[40] H.Z. Shi, F.J. Quintero, J.M. Pardo, J.K. Zhu, The putative plasma membrane Na^+/H^+ Antiporter SOS1 controls long-distance Na^+ transport in plants, Plant Cell 14 (2002) 465–477.

[41] R. Olías, Z. Eljakaoui, J. Li, P.A. De-Morales, M.C. Marín-Manzano, J.M. Pardo, A. Belver, The plasma membrane Na^+/H^+ antiporter SOS1 is essential for salt tolerance in tomato and affects the partitioning of Na^+ between plant organs, Plant Cell Environ. 32 (2009) 904–916.

[42] D. Golldack, F. Quigley, C.B. Miehalowski, U.R. Kamasani, H.J. Bohnert, Salinity stress-tolerant and -sensitive rice (*Oryza sativa* L.) regulate AKT1-type potassium channel transcripts differently, Plant Mol. Biol. 51 (2003) 71–81.

[43] S.M. Wang, J.L. Zhang, T.J. Flowers, Low-affinity Na^+ uptake in the halophyte *Suaeda maritima*, Plant Physiol. 145 (2007) 559–571.

[44] Q. Guo, P. Wang, Q. Ma, J.L. Zhang, A.K. Bao, S.M. Wang, Selective transport capacity for K^+ over Na^+ is linked to the expression levels of *PtSOS1* in halophyte *Puccinellia tenuiflora*, Funct. Plant Biol. 39 (2012) 1047–1057.

[45] H.J. Yuan, Q. Ma, G.Q. Wu, P. Wang, J. Hu, S.M. Wang, ZxNHX controls Na^+ and K^+ homeostasis at the whole-plant level in *Zygophyllum xanthoxylum* through feedback regulation of the expression of genes involved in their transport, Ann. Bot. 115 (2015) 495–507.

[46] R.J. Davenport, A. Muñoz-Mayor, D. Jha, P.A. Essah, A. Rus, M. Tester, The Na^+ transporter AtHKT1;1 controls retrieval of Na^+ from the xylem in *Arabidopsis*, Plant Cell Environ. 30 (2007) 497–507.

[47] T. Horie, J. Motoda, M. Kubo, H. Yang, K. Yoda, R. Horie, W.Y. Chan, H.Y. Leung, K. Hattori, M. Konomi, Enhanced salt tolerance mediated by AtHKT1 transporter-induced Na^+ unloading from xylem vessels to xylem parenchyma cells, Plant J. 44 (2005) 928–938.

[48] F. Hauser, T. Horie, A conserved primary salt tolerance mechanism mediated by HKT transporters: a mechanism for sodium exclusion and maintenance of high K^+/Na^+ ratio in leaves during salinity stress, Plant Cell Environ. 33 (2010) 552–565.

[49] P. Berthomieu, G. Conejero, A. Nublat, W.J. Brackenbury, C. Lambert, C. Savio, N. Uozumi, S. Oiki, K. Yamada, F. Cellier, Functional analysis of *AtHKT1* in *Arabidopsis* shows that Na^+ recirculation by the phloem is crucial for salt tolerance, EMBO J. 22 (2003) 2004–2014.

[50] T. Horie, F. Hauser, J.I. Schroeder, HKT transporter-mediated salinity resistance mechanisms in *Arabidopsis* and monocot crop plants, Trends Plant Sci. 14 (2009) 660–668.

[51] H.X. Xu, X.Y. Jiang, K.H. Zhan, X.Y. Cheng, X.J. Cheng, J.M. Pardo, D. Cui, Functional characterization of a wheat plasma membrane Na^+/H^+ antiporter in yeast, Arch. Biochem. Biophys. 473 (2008) 8–15.

[52] D.H. Oh, E. Leidi, Q. Zhang, Y.Z. Li, W.Y. Ma, D.J. Yun, R.A. Bressan, H.J. Bohnert, Loss of halophytism by interference with SOS1 expression, Plant Physiol. 151 (2009) 210–222.

[53] G. Scoles, P. Maughan, T. Turner, C. Coleman, D. Elzinga, E. Jellen, J. Morales, J. Udall, D. Fairbanks, A. Bonifacio, Characterization of salt overly sensitive 1 (SOS1) gene homoeologs in quinoa (*Chenopodium quinoa* wild.), Genome 52 (2009) 647–657.

[54] R. Davenport, R.A. James, A. Zakrisson-Plogander, M. Tester, R. Munns, Control of sodium transport in durum wheat, Plant Physiol. 137 (2005) 807–818.

[55] B. Wang, R.J. Davenport, V. Volkov, A. Amtmann, Low unidirectional sodium influx into root cells restricts net sodium accumulation in *Thellungiella halophila*, a salt-tolerant relative of *Arabidopsis thaliana*, J. Exp. Bot. 57 (2006) 1161–1170.

[56] H.J. Kronzucker, M.W. Szczerba, L.M. Schulze, D.T. Britto, Non-reciprocal interactions between K^+ and Na^+ ions in barley (*Hordeum vulgare* L.), J. Exp. Bot. 59 (2008) 2793–2801.

[57] A. Amtmann, D. Sanders, Mechanism of Na^+ uptake by plant cells, Adv. Bot. Res. 29 (1998) 75–112.

[58] P.H. Buschmann, R. Vaidynathan, W. Gassmann, J.I. Schroeder, Enhancement of Na^+ uptake currents, time dependent inward-rectifying K^+ channel currents, and K^+ channel transcripts by K^+ starvation in wheat root cells, Plant Physiol. 122 (2000) 1387–1398.

[59] N. Uozumi, E.J. Kim, F. Rubio, T. Yamaguchi, S. Muto, A. Tsuboi, E.P. Bakker, T. Nakamura, J.I. Schroeder, The *Arabidopsis HKT1* gene homolog mediates inward Na^+ currentsin *Xenopuslaevis oocytes* and Na^+ uptake in *Saccharomy cescerevisiae*, Plant Physiol. 122 (2000) 1249–1259.

[60] A. Rus, S. Yokoi, A. Sharkhuu, M. Reddy, B. Lee, T.K. Matsumoto, H. Koiwa, J.K. Zhu, R.A. Bressan, P.M. Hasegawa, AtHKT1 is a salt tolerance determinant that controls Na^+ entry into plant roots, PNAS 98 (2001) 14150–14155.

[61] T. Horie, A. Costa, T.H. Kim, M.J. Han, R. Horie, H.Y. Leung, A. Miyao, H. Hirochika, G. An, J.I. Schroeder, Rice OsHKT2;1 transporter mediates large Na^+ influx component into K^+-starved roots for growth, EMBO J. 26 (2007) 3003–3014.

[62] L.H. Wegner, A.H. De Boer, Properties of two outward-rectifying channels in root xylem parenchyma cells suggest a role in K^+ homeostasis and longdistance signaling, Plant Physiol. 115 (1997) 1707–1719.

[63] F. Gaymard, G. Pilot, B. Lacombe, D. Bouchez, D. Bruneau, J. Boucherez, N. Michaux-Ferriere, J.B. Thibaud, H. Sentenac, Identification and disruption of a plant shaker-like outward channel involved in K^+ release into the xylem sap, Cell 94 (1998) 647–655.

[64] L. Shabala, T.A. Cuin, I.A. Newman, S. Shabala, Salinity induced ion flux patterns from the excised roots of *Arabidopsis sos* mutants, Planta 222 (2005) 1041–1050.

[65] S. Shabala, V. Demidchik, L. Shabala, T.A. Cuin, S.J. Smith, A.J. Miller, J.M. Davies, I.A. Newman, Extracellular Ca^{2+} ameliorates NaCl-induced K^+ loss from Arabidopsis root and leaf cells by controlling plasma membrane K^+-permeable channels, Plant Physiol. 141 (2006) 1653–1665.

[66] U. Anschütz, D. Becker, S. Shabala, Going beyond nutrition: regulation of potassium homoeostasis as a common denominator of plant adaptive responses to environment, J. Plant Physiol. 171 (2014) 670–687.

[67] Q. Wang, C. Guan, S.M. Wang, Coordination of AtHKT1;1 and AtSOS1 facilitates Na^+ and K^+ homeostasis in *Arabidopsis thaliana* under salt stress, J. Plant Biol. 57 (2014) 282–290.

[68] H.Z. Shi, B.H. Lee, S.J. Wu, J.K. Zhu, Overexpression of a plasma membrane Na^+/H^+ antiporter gene improves salt tolerance in *Arabidopsis thaliana*, Nat. Biotechnol. 21 (2003) 81–85.

[69] T.J. Flowers, S.A. Flowers, M.A. Hajibagheri, A.R. Yeo, Salt tolerance in the halophytic wild rice, *Porteresia coarctata Tateoka*, New Phytol. 114 (1990) 675–684.

[70] J. Rozema, H. Gude, G. Pollak, An ecophysiological study of the salt secretion of four halophytes, New Phytol. 89 (1981) 201–217.

[71] V. Amarasinghe, L. Watson, Comparative ultrastructure of microhairs in grasses, Bot. J. Linn. Soc. 98 (1988) 303–319.