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Full Length Article

Salt Stress Changes the Ion Homeostasis via Differential K^+ Uptake Ways in Salt-Sensitive/Tolerant Wheat (*Triticum aestivum*) Varieties

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Abstract

The capacity of K^+ acquisition and retention in wheat (*Triticum aestivum* L.) roots is related to salt tolerance. Salt-tolerant 'C6005' and salt-sensitive 'AK58' wheat cultivars were used with combined treatment of NaCl stress and K^+ transport blockers to study the effect of salt stress on ion homeostasis. NaCl induced an apparent decline of K^+/Na^+ ratios in two wheat cultivars. The K^+/Na^+ ratio in 'C6005' was higher than that in 'AK58'. Inhibitors TEA (Tetraethylammonium chloride), NEM (N-ethylmaleimide) and Ba $(NO_3)_2$ all influenced the transport and accumulation of K^+ and Na^+ in root and leaf tissues, and NEM exhibited the most obvious effects. Combined NEM and NaCl treatment induced the highest K^+ efflux and the value of K^+ efflux in 'AK58' was higher than that in 'C6005'. Level of 250 m*M* NaCl increased the activity of K^+/H^+ transporters in 'C6005' but decreased that in 'AK58'. NaCl decreased the K^+_{in} currents in root protoplast and the amplitude of K^+_{in} currents in 'AK58' was more obvious than that in 'C6005'. NaCl stress up-regulated the expression of *HAK5* in roots of both cultivars, the trend in 'C6005' with combined NEM and NaCl treatment at 48 h was most evident. This work concluded that the K^+ transporters may be more important way to absorb K^+ compared to other two ways. The regulation of *HAK5* expression at transcript level could play a key role in the ion homeostasis in wheat roots. © 2019 Friends Science Publishers

Keywords: Wheat; Salt stress; K^+ uptake; Ion homeostasis

Introduction

Soil salinity is regarded as a worldwide agricultural threat, limiting growth and yield of many crops. Due to unplanned application of fertilizers and irrigation, salinization is getting more and more serious issue (Munns and Tester, 2008; Fahad *et al*., 2015). As one of the major abiotic factors, effects of salt stress on physiology and metabolism of plants have been studied (Widodo *et al*., 2009). It is universally known that hypersaline environments have dual influence on plants by ionic imbalance and water loss in root cells under high osmotic pressure (Zhu, 2003).

From the viewpoint of plant evolution, some sophisticated mechanisms formed to adapt to salt stress in some species. For a long time, Na^+ is the focus, including how Na^+ is transported via plasma membrane and how Na^+ is extruded or compartmentalized by plant cells (Vera-Estrella *et al*., 2005). Recently, the importance of K^+ retention in conferring salt tolerance had been emphasized in many crops (Cuin *et al*., 2008; Shabala and Pottosin, 2014; Zhang *et al*., 2015b). Under salt stress, the uptake of many essential ions decreases obviously, essentially K^+ which has similar chemical characteristics with Na^+ (Zepedajazo *et al*., 2008). Salt stress induced oxidative stress, lipid peroxidation and lower yield in wheat, potassium supplementation could alleviate the bad effects of salinity stress (Ahanger and Agarwal, 2017). K^+ has been involved in so many physiological activities, such as enzyme activation, electrical signaling and osmoregulation, photosynthesis, transport/translocation of assimilation products (Cuin *et al*., 2008).

Usually, K^+ concentration in cytoplasmic was kept up to 100 m*M* approximately, well above that at the rhizosphere soil, 0.1–1 m*M* (Wang and Wu, 2013). K^+ is acquired by specific K^+ transport systems located in roots, including K^+ channels and K^+ transporters and nonselective cation channels (NSCCs), to maintain the K^+ concentration gradient on both sides of membrane (Dreyer and Uozumi, 2011). Based on the studies on *A. thaliana*, three systems play different roles: NSCCs occupy a position of importance at high K^+ concentration (>10 m*M*); the K^+ channel AKT1 dominates K^+ uptake at intermediate K^+ concentrations (1 m*M*); AKT1 channels and K^+ transporter (HAK5) team up for K^+ transport at low concentration (100 μM); Only K^+ transporter (HAK5) may play a role on K^+ acquisition at extremely low concentration

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(<10 μM) (Nieves-Cordones *et al*., 2014).

The K^+ channel and NSCCs, as passive transport, have been supposed as low-affinity K^+ uptake, the activity of channels depends on membrane hyperpolarization and the H^+ gradient and driving force from H^+-ATPase. AKT and KAT, two members of Shake-like channel family, were deemed to absorb K^+ in *Arabidopsis thaliana* firstly (Yao *et al*., 2017). As an inward rectifying channel, *AKT1* expression correlated with K^+ uptake. Under osmotic/drought stress, *OsAKT1* over expression increased the content of K^+ in roots and improved the osmotic and drought stress tolerance in rice (Ahmad *et al*., 2016). The transcriptional down regulation of *OsAKT1* in rice roots had already been proved under salt stress and Patch-clamp studies on rice root protoplasts identified a significant decrease of inward K^+ currents (Fuchs and Hedrich, 2005). It seems that *OsAKT1* mediate K^+ uptake under some abiotic stress, but salt stress maybe specific on the expression of *OsAKT1*. K^+ starvation could up-regulate the *TaAKT1* mRNA levels and increase the time-dependent inward-rectifying K^+ channel currents (Buschmann *et al*., 2000), but the effect of salt on *TaAKT1* is not studied deep enough so far.

As an active transport way, K^+ transporter play a key role on root K^+ uptake under very low concentration of external K^+ (Brauer *et al*., 2016). Research on *A. thaliana* showed that the high-affinity K^+ transporter HAK5 was induced up-regulated when environment conditions, especially external K^+, were limited (Rubio *et al*., 2014). Salt stress induced the hyperpolarization of plasma membrane which further propels K^+ efflux. *OsHAK21* and *OsHAK1*, members of HAK family, showed a significant inducement expression under high salinity in some rice tissues, especially root epidermises. *Oshak1* and *Oshak21* knockout mutants showed a dramatic reduction in K^+ concentration and increment in Na^+ concentration (Chen *et al*., 2015a; Shen *et al*., 2016). Another high-affinity K^+ transporter HKT1, cloned from wheat firstly, was proved to regulate K^+-Na^+ cotransport. Tahkt1 may mediate Na^+ acquirtion from environment under salt stress, and retrieve Na^+ from the xylem vessels in the roots. As a result, plant could maintain a well-balanced K^+/Na^+ equilibrium in shoots for salinity tolerance (Byrt *et al*., 2014). The expression of *Tahkt1;5* in wheat leaves were earlier and higher than that in roots under salt stress and were higher in salt-tolerant genotypes than that in salt-sensitive genotypes (Babgohari *et al*., 2013).

In this study, the K^+ and Na^+ content, and K^+ efflux under salt stress in wheat varieties with different tolerance to salinity stress were compared firstly; then the contribution of different K^+ transport systems to ion homeostasis were studied by virtue of corresponding inhibitors. Finally, the primers for genes related to K^+ channels and K^+ transporters were designed and the comparative gene expression analysis was expected to find their correlation with salt resistance.

Materials and Methods

Wheat Seedling and Growth Conditions

Mature seeds of wheat (*Triticum aestivum* L.) cultivars "Cang 6005 (C6005)" and "Aikang 58 (AK58)" were used. Equally-sized healthy seeds were chosen and disinfected in 0.1% $HgCl_2$ for 15 min and soaked in distilled water for 12 h, then allowed to germinate in moistened gauze in the dark for 12 h, until the primary roots reaching about 1 cm in length. Then all the seedlings were grown in a growth chamber under the normal condition (23°C/18°C with 16 h light/8 h dark cycles). The Hoagland's solution was used as nutrients and refreshed every 2 days. 10 d age seedlings with two leaves were exposed to 250 m*M* NaCl stress liquids made up with Hoagland's nutrient solution, with or without K^+ transport inhibiters (Ding *et al*., 2006; Zhang *et al*., 2015a). The stress treatment was designed with 3 replications and lasted for 7 d.

Determination of Ion Content

Wheat roots from each treatment were harvested separately and cleaned up in flowing ddH_2O. The roots were dried in forced-air oven at 105°C for 30 min, followed by 12 h at 65°C. The completely dried roots were grinded by ball mill (model: MM400, Retsch company, Germany). K^+ and Na^+ content in samples were measured by the inductively coupled plasma atomic emission spectrometry (ICP-AES).

K^+ Flux Measurement

Roots from wheat seedlings were used for K^+ flux measurement with non-invasive micro-test technology using NMT100 Series (Younger USA LLC, Amherst, MA01002, USA) at the younger USA (Xuyue, Beijing) NMT Service Center. Before measurement, root was exposed to 250 m*M* NaCl for 0 or 30 min in the NaCl treated group and exposed in TEA/NEM/$Ba(NO_3)_2$ for 30 min in inhibitor treatment group, then followed by 250 m*M* NaCl treatment. The following net K^+ flux measurement was as described previously (Chen *et al*., 2007). At least six individual roots from different seedlings were used for every independent experiment.

Isolation and Purification of Wheat Root Plasma Membranes

Root plasma membrane vesicles were extracted and purified according to the methods of Faraday and Spanswick (1992), Ahn *et al*. (2001) and Shen *et al*. (2006) with some modification. About 5 g fresh roots were pestle in liquid nitrogen and then transferred to a 50 mL EP tube, 10 mL extracting buffer (300 m*M* sucrose, 50 m*M* Hepes, 8 m*M* EGTA, 2 m*M* PMSF, 4 m*M* DTT, 1.5% PVPP, 0.2% BSA, pH 7.0 (Tris)) were added and mixed well. The samples were centrifuged in a swinging bucket rotor at 10000 × g for

20 min in 4°C and the supernatants were further centrifuged at 50000 × g for 35 min in 4°C, the lower sediments were resuspended in 1 mL suspension buffer (300 m*M* sucrose, 5 m*M* KH_2PO_4, 5 m*M* KCl, 0.1 m*M* EDTA and 1 m*M* DTT) and loaded on two-phase solution (6.2% Dextran T500, 6.2% PEG3350 in suspension buffer), and then centrifuged at 2500 × g for 10 min in 4°C. The supernatants were separated and diluted with 5-fold diluting buffer (300 m*M* sucrose, 5 m*M* Hepes, 1 m*M* DTT, pH 7.0 (Tris)), then centrifuged at 80000 × g for 40 min in 4°C. The lower sediments were resuspended with diluting buffer and stored at -80°C. The concentrations of plasma membranes proteins were determined with BSA as the standard based on Bradford (Jones *et al*., 1989).

Examination of Plasma Membrane H^+-ATPase and H^+-PPase Activity

A 50 μL plasma membranes was added to a 0.5 mL volume of reaction mixtures (100 m*M* Hepes, 20 m*M* $MgSO_4$, 500 m*M* KNO_3, 5 m*M* NaN_3, 1 m*M* $(NH_4)_6Mo_7O_{24}$•$4H_2O$), 50 μL of 20 m*M* ATP-Tris or Na_4PPi (pH 7.5) was used as the substrate. Reactions were proceeded for 20 min at 37°C and stopped with boiling water bath for 10 min. A 0.2 mL above reaction solution was added to 5 mL color developing agent (0.5% ascorbic acids in 7.5N antimony molybdenum sulfate, freshly made) for 15 min in dark, and then its absorbance at 700 nm was measured. The activity of H^+-ATPase or H^+-PPase was expressed as the μM of Pi released from ATP-Tris or Na_4PPi per mg plasma membranes in 1 min at 37°C.

Measurement of K^+/H^+ Transport Activity

According to the methods mentioned by Lin *et al*. (2014) and Zhang *et al*. (2015a) with minor modification, K^+/H^+ transport activity was measured by a spectrofluorometric (F-4600, Hitachi, Tokyo) with excitation at 495 nm and emission at 540 nm, slits 2.5 nm. The reaction buffer contained 250 m*M* sucrose, 100 m*M* KNO_3, 1 m*M* NaN_3, 3 m*M* $MgSO_4$, 20 m*M* Tricine, 5 μ*M* acridine orange and adjusted pH 7.5 with Tris. The reaction started in dark by adding ATP-Tris (3 m*M*) and the change of fluorescence was recorded as ΔF. K-gluconate (final concentration 20 m*M*) was added when ΔF reaches its equilibrium. The resumptive value of fluorescence was recorded as ΔF'. K^+/H^+ transport activity was measured as $\Delta F'/\Delta F.\mu g^{-1}.min^{-1}$.

Protoplast Isolation and Electrophysiology

Young roots about 1 cm in length were picked up for protoplast preparation according to Schachtman and Terry (1991). Whole-cell and single-channel patch-clamp experiments were carried out using an Axopatch-200B (Axon Instruments, Foster City, CA, USA) as described by Buschmann *et al*. (2000). Electrodes were pulled with glass capillaries and filled with pipette solution (100 m*M* $C_6H_{11}KO_7$, 0.1 m*M* $CaCl_2.2H_2O$, 2 m*M* $MgCl_2.6H_2O$, 10 m*M* Hepes, 2 m*M* $ATP.Na_2$, 1.1 m*M* EGTA, 550 m*M* D-manitol, adjust pH7.2 with Tris). The appropriate resistance of electrodes in bath solution retains 10~20 MΩ. Patching of protoplasts was developed in a flow chamber within 1 mL bath solution, and instantaneous current was recorded continuously for 3 min designed in advance. K^+_{in} currents were recorded when 1 *M* NaCl and 1 *M* TEA (tetraethylammonium chloride) were added to the chamber.

Gene Relative Expression Analysis

The roots from seedlings with two leaves were exposed to K^+ transport inhibitors (TEA, NEM and Ba $(NO_3)_2$) or not for 12 h firstly, then they were divided into two group, with 0 or 250 m*M* NaCl was added up respectively. Roots were collected for RNA isolation at 4, 24 and 48 h after salt treatment. The total RNA was extracted and reverse transcribed as described by Li *et al*. (2015). The cDNA was used as the template in the following real-time quantitative PCR on the 7500/7500 Fast Real-Time PCR System (Applied Biosystems, CA, USA). The primers of *TaHAK5*, *TaHKT1*, *TaAKT1*, and *TaKAT2* were designed by means of homology search and blast between *A. thaliana* and wheat with DNAman, their base sequences were as follows: *TaHAK5* forward: 5'-CAGAGCCTAGGTGTGGTTTATG-3', reverse: 5'-GTCCTTGATCCCACCAGTAAAG-3'; *TaHKT1* forward: 5'- CTGCCGTATCAGTGGATATGTT-3', reverse: 5'-TAGCAATGGCAAGGAAGTAGG-3'; *TaAKT1* forward: 5'-GGTTGCTGCTTGAATTGTATCC-3', reverse: 5'-ACCTTGGACTGACTGCTTATTT-3'; *TaKAT2* forward: 5'-CAACGAGCACAAGCAAGAAC-3', reverse: 5'-ATCTCTAGGAGCAAGAGCAAAC-3'; *Actin* forward: 5'-GAATTCACGAGACCACCTACAA-3', reverse: 5'-TGCTCATACGGTCAGCAATAC-3'. *Actin* was used as an endogenous control gene. The qRT-PCR reactions were performed with SYBR Green master mix (GenStar Biosolutions, Beijing, China) in a 20 μL volume. The relative quantification of gene expression was calculated using the relative Ct method and presented as $2^{-\Delta\Delta CT}$.

Statistical Analysis

Analysis of variance of data was performed with SPSS statistics 19.0 software. For statistical analysis, 1-way analysis of variance and a 2-tailed t test (α=0.05) were used to determine the significance at $P < 0.05$.

Results

K^+ (Na^+) Content and K^+/Na^+ Ratio

Salt-tolerant variety 'C6005' had a generally higher K^+ content and K^+/Na^+ ratio and less Na^+ content than 'AK58'

Table 1: Effects of NaCl and blockers on K^+ and Na^+ content in wheat leaves and root tissues

Treatment		K^+(mmol.g^{-1})		Na^+(mmol.g^{-1})		K^+/Na^+ (%)	
		roots	leaves	roots	leaves	roots	leaves
AK58	CK	4.03±0.01	6.84±0.02	0.76±0.02	0.21±0.01	5.32±0.05	32.81±0.17
	NaCl	1.29±0.01	5.55±0.04	2.93±0.01	8.50±0.03	0.44±0.01	0.65±0.01
	TEA	3.89±0.02	6.18±0.02	0.71±0.01	0.28±0.01	5.45±0.06	22.18±0.05
	NEM	1.97±0.01	4.41±0.02	0.29±0.01	0.29±0.01	6.81±0.02	15.30±0.04
	$Ba(NO_3)_2$	3.33±0.01	6.74±0.02	0.65±0.01	0.22±0.01	5.13±0.02	30.98±0.09
	TEA+NaCl	1.60±0.01	4.07±0.02	2.73±0.02	4.12±0.02	0.58±0.01	0.99±0.01
	NEM+NaCl	0.52±0.01	5.21±0.01	1.84±0.02	5.99±0.02	0.29±0.01	0.87±0.01
	$Ba(NO_3)_2$+NaCl	1.51±0.02	5.33±0.01	3.27±0.02	4.26±0.02	0.46±0.01	1.25±0.01
C6005	CK	4.47±0.01	7.29±0.02	0.56±0.01	0.20±0.01	7.97±0.16	35.79±0.11
	NaCl	2.37±0.02	5.18±0.06	2.84±0.02	6.29±0.03	0.83±0.02	0.82±0.01
	TEA	3.33±0.01	6.09±0.01	0.54±0.01	0.22±0.01	6.21±0.10	27.87±0.15
	NEM	2.33±0.01	4.95±0.02	0.25±0.01	0.27±0.01	9.23±0.02	18.11±0.16
	$Ba(NO_3)_2$	3.99±0.02	6.81±0.02	0.73±0.01	0.20±0.01	5.43±0.09	34.28±0.32
	TEA+NaCl	2.11±0.01	5.07±0.03	2.78±0.01	6.01±0.03	0.76±0.02	0.84±0.01
	NEM+NaCl	1.02±0.01	4.40±0.02	1.80±0.01	3.46±0.02	0.56±0.01	1.27±0.01
	$Ba(NO_3)_2$+NaCl	1.64±0.02	5.74±0.02	2.71±0.01	6.69±0.03	0.60±0.01	0.86±0.01

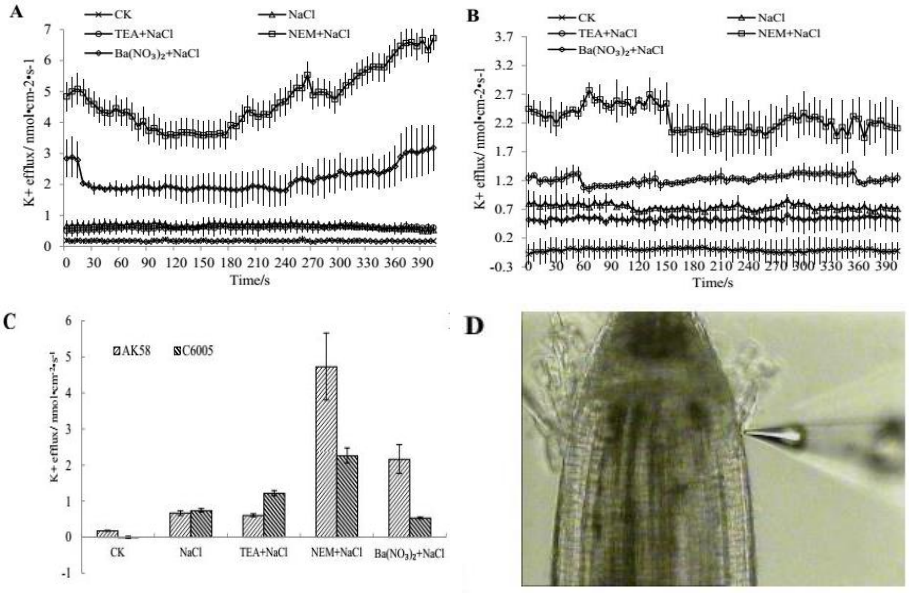

Fig. 1: Transient K^+ fluxes in response to blockers pretreatment and NaCl treatment and their mean value of transient K^+ fluxes. Note: Each value is the Mean ±SE (n=6) (A): AK58 (B): C6005 (C): Mean value of K^+ fluxes. In all NMT measurements, the sign convention is efflux positive (D): Root tip was being measured by microelectrode

in comparative treatment (Table 1), especially under NaCl stress condition, the comparative data were +83.7% (K^+), +88.6% (K^+/Na^+ ratio) and -3.1% (Na^+) in roots, respectively. A significant decline in K^+ and K^+/Na^+ ratio was observed after 7 d 250 m*M* NaCl treatments, but the data for 'C6005' was less than that for 'AK58', being -47% *vs.* -68% for root K^+ content and -89.6% *vs.* -91.7% for K^+/Na^+ ratio, respectively, compared with the control groups. Inhibitors TEA, NEM and $Ba(NO_3)_2$ all influenced the accumulation of K^+ and Na^+ in root and leaf tissues, but only NEM exhibited the most obvious effects. Compared with TEA or $Ba(NO_3)_2$, single NEM treatment induced a more obvious decline of K^+ content in the root and leaf tissues and Na^+ content in root.

K^+ Fluxes in Root Hairs Cells

The transient K^+ flux was exhibited in Fig. 1. K^+ efflux in the control groups were very small and 250 m*M* NaCl pretreatment for 30 min increased the K^+ efflux greatly in both wheat cultivars. The mean values of transient K^+ fluxes were not different statistically between the two cultivars in both control and pure NaCl stress condition (Fig. 1C). TEA pretreatment plus 30 min NaCl treatment significantly increased K^+ efflux in 'C6005', but not in 'AK58', compared to its single NaCl treatment. NEM pretreatment plus NaCl treatment for 30 min significantly increased K^+ efflux ($P<0.05$) in both wheat cultivars compared to the other groups and the

increments were generally two and six times for 'C6005' and 'AK58', respectively, compared to their relative pure NaCl treatment. $Ba(NO_3)_2$ pretreatment significantly increased K^+ efflux in 'AK58', but not in 'C6005', when were compared with their relative pure NaCl treatment.

H^+-ATPase and H^+-PPase Activity in Plasma Membrane of Roots

The activity of H^+-ATPase in all groups of NaCl, TEA/NEM/$Ba(NO_3)_2$ treated wheat significantly reduced after 7 d treatment compared with respective controls, excepting that TEA treatment in 'C6005', Ba $(NO_3)_2$ treatment in 'C6005' and 'AK58' (Fig. 2). When two cultivars compared, NaCl induced a decline in H^+-ATPase activity in 'C6005' (58.2%) was lower than that in 'AK58' (76.8%). NEM treatment resulted in a much more decrease of H^+-ATPase activity in 'C6005' (82.03%) than 'AK58' (58.69%). $Ba(NO_3)_2$ showed inconspicuous effects on H^+-ATPase activity. Different from that of H^+-ATPase, NaCl stress induced a 17.0% increase in H^+-PPase activity in 'AK58' and a 26.4% decrease in 'C6005', respectively, relative to their normal control (Fig. 3). TEA and NEM showed a reverse effects on H^+-PPase activity, TEA treatment increased the H^+-PPase activity by +19.2% ('C6005') and +36.1% ('AK58'); whereas NEM treatment decreased it by -62.9% ('C6005') and -81.0% ('AK58'), respectively, compared to their respective controls; both varieties showed similar trends. But different influence of Ba $(NO_3)_2$ treatment on H^+-PPase activity were shown in the two varieties, being +17.3% increment in 'C6005' and -30.9% decrement in 'AK58', respectively, relative to their normal controls.

K^+/H^+ Transporter Activities in Root Plasma Membrane

Treatment with 250 m*M* NaCl for seven days treatment significantly increased or decreased the activities of K^+/H^+ transport in 'C6005' or 'AK58', respectively, with respect to their relative controls (Fig. 4). NEM treatment had negative effect on K^+/H^+ transport activity and of the same tendency in the two varieties. Compared with NaCl treatment group, an extremely significant reduction of K^+/H^+ transport activity appeared in NaCl plus NEM treatment group, and was more apparent in 'C6005' than in 'AK58'.

K^+ Channel Activities in Protoplast of Root Hair Cells

The K^+_{in} currents were decreased with the voltage gradient reduction and restrained when NaCl were added to the flow chamber (Fig. 5). K^+_{in} currents at 120 mV voltage were decreased by 41.5 and 23.6% under salt tress, compared with relative controls, for 'AK58' and 'C6005', respectively. The K^+_{in} currents of 'AK58' were more susceptible to salt stress than that of 'C6005' and were suppressed intensely when TEA (final concentration 10 m*M*) was continued to add to the flow chamber.

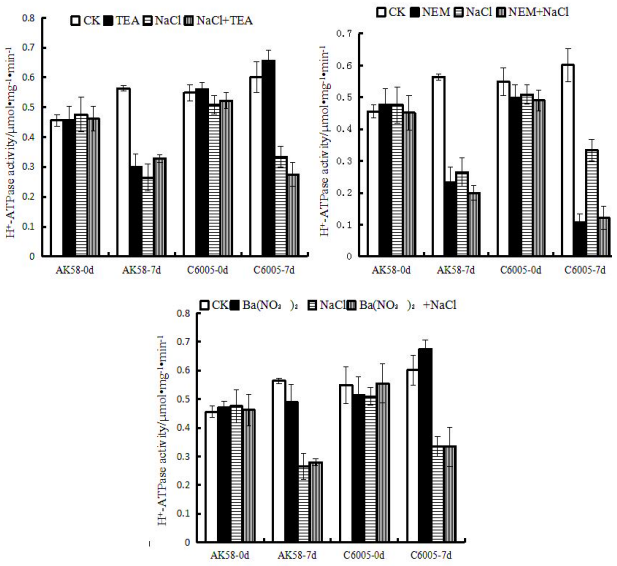

Fig. 2: Effects of NaCl and blockers on the activities of H^+-ATPase in plasma membrane of salt-tolerant and sensitive wheat roots

Note. Each value is the Mean ±SE (n=3). Different lowercases show the significant difference (P<0.05)

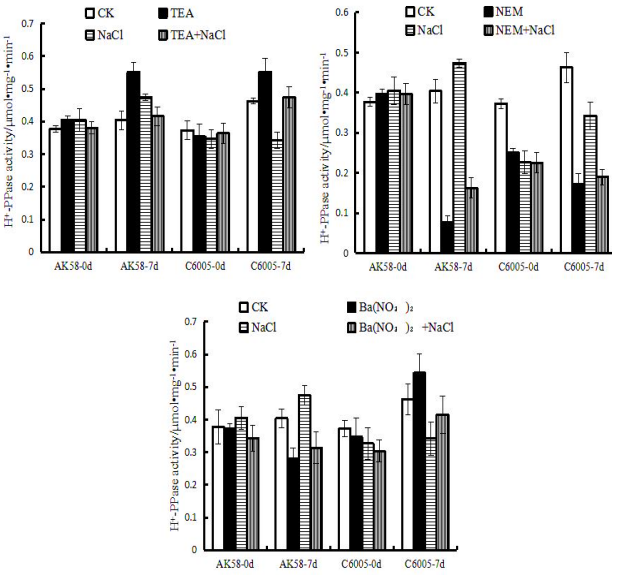

Fig. 3: Effects of NaCl and blockers on the activities of H^+-PPase in plasma membrane of salt-tolerant and sensitive wheat roots. Note: Each value is the Mean ±SE (n=3). Different lowercases show the significant difference (P<0.05)

Expression of Genes under NaCl and Inhibitors Treatment

The expression of HAK5 was up-regulated significantly at 24 and 48 h after 250 m*M* NaCl shock, and the expression

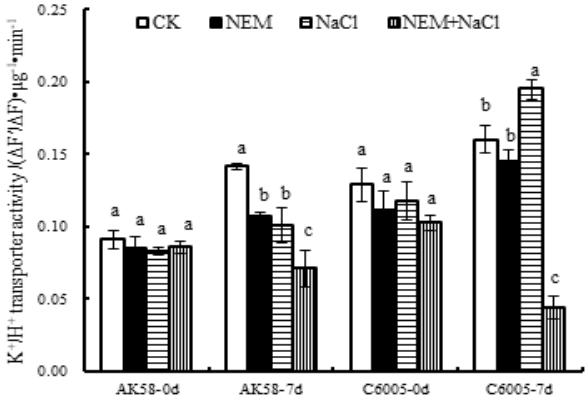

Fig. 4: Effects of NaCl and blockers on the activities of K^+/H^+ transporters in plasma membrane of salt-tolerant and sensitive wheat roots. Note: Each value is the Mean ±SE (n=3). Different lowercases show the significant difference (P<0.05)

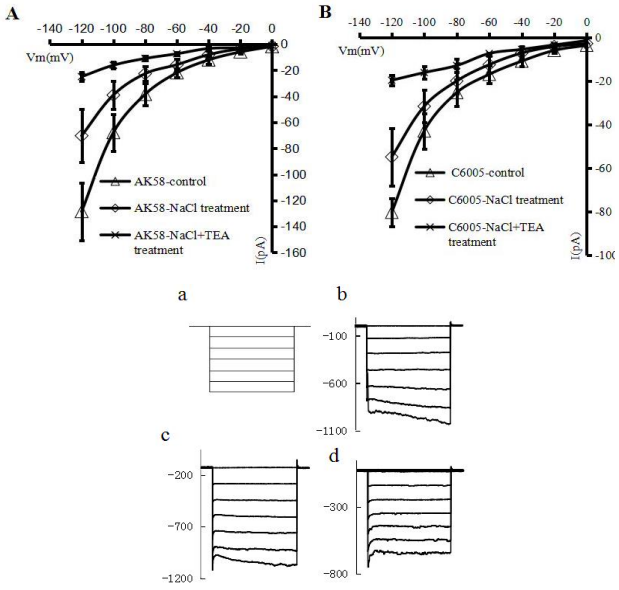

Fig. 5: Whole-cell K^+ in currents in the plasma membrane of the root protoplast isolated from two wheat cultivars were recorded under NaCl stress. Note: The whole-cell currents evoked by an increasingly voltage, with pulses from -120mV to 0mV (a), the real time current of control group (b), NaCl treatment group (c) and TEA treatment (d). Note. Each value is the Mean ±SE (n=3). Different lowercases show the significant difference (P<0.05)

levels were higher in 'C6005' than in 'AK58' (Fig. 6A). TEA and NEM both had superimposed effects with NaCl treatment on HAK5 expression, and a striking up-regulation about 189-fold was exhibited in NEM and NaCl combined treatment of 'C6005', relative to its normal control group. The data of HKT1 expression were less than that of HAK5 and were decreased in some degree by salt stress, excepting that 'AK58' at 48 h. (Fig. 6B). TEA and NaCl combined treatment significantly down-regulated the expression of HKT1 at 4 and 48 h. However, NEM and NaCl combined treatment significantly up-regulated the expression of HKT1 at 24 h. The expression of AKT1 were reduced in both cultivars by NaCl stress, excepting that of 'AK58' at 48 h, and the descend range in cultivar 'C6005' were more remarkable than that in 'AK58' (Fig. 7A). TEA and NaCl combined treatment significantly down-regulated the expression of AKT1 in 'C6005' at 48 h, relative to its TEA treatment. However, combined NEM and NaCl treatment had no obvious influence on AKT1 expression, compared with relative NEM treatment group. In contrast to AKT1, the expression levels of KAT2 were increased sharply by NaCl stimulus with a peak value at 4 h from the onset of NaCl stress (Fig. 7B). The amplitude of upregulation was more remarkable in 'C6005' than in 'AK58'. TEA/NaCl combined and NEM/NaCl combined treatments both enhanced the up-regulated expression of KAT2, relative to their respect inhibitor treatment, excepting that 'AK58' in combined TEA/NaCl treatment at 48 h and that 'C6005' in combined NEM/NaCl treatment at 24 h.

Discussion

The maintenance of a high cytosolic K^+/Na^+ ratio plays a critical role on the adaptive response to salinity in plants (Shabala and Cuin, 2008). Unexpected abundance Na^+ causes detrimental effects on ion homeostasis (Deinlein *et al*., 2014), NaCl-induced K^+ imbalance was generally considered as the main damage from salt stress, whilst the overlooked ability of retaining K^+ in plats maybe more important in regulating ion homeostasis and alleviating salt stress than did Na^+ exclusion (Zepedajazo *et al*., 2008). Increased K^+ influx and decreased K^+ efflux in roots were both helpful to maintain the intracellular K^+ concentration (Yu *et al*., 2016). Salt-tolerant 'C6005' showed a better ability of K^+ retention by virtue of root K^+ content and K^+/Na^+ ratio than did 'AK58' under 250 m*M* NaCl stress, which was consistent with that in wheat cultivars 'DK961' and 'JN17' (Zheng *et al*., 2008), but was not verified by K^+ fluxes measurement, where the mean values of transient K^+ fluxes were not different statistically between the two cultivars in both control and NaCl stress condition. This may be attributed to the different time length of salt stress. High plant have own K^+ transporters and channels for K^+ uptake from the soil against the K^+ concentration gradient (Wang and Wu, 2013). This study showed that inhibitors TEA, NEM and Ba $(NO_3)_2$ all influence the transport and accumulation of K^+ and Na^+ in root and leaf tissues, but only NEM treatment induced a more obvious decline of K^+ content in the root and leaf tissues and Na^+ content in root. Patch-clamp showed that K^+_{in} currents at 120 mV voltage were decreased by 41.5% ('AK58') and 23.6% ('C6005') under salt tress, compared with relative controls, respectively, indicating that 'AK58' was more susceptible to

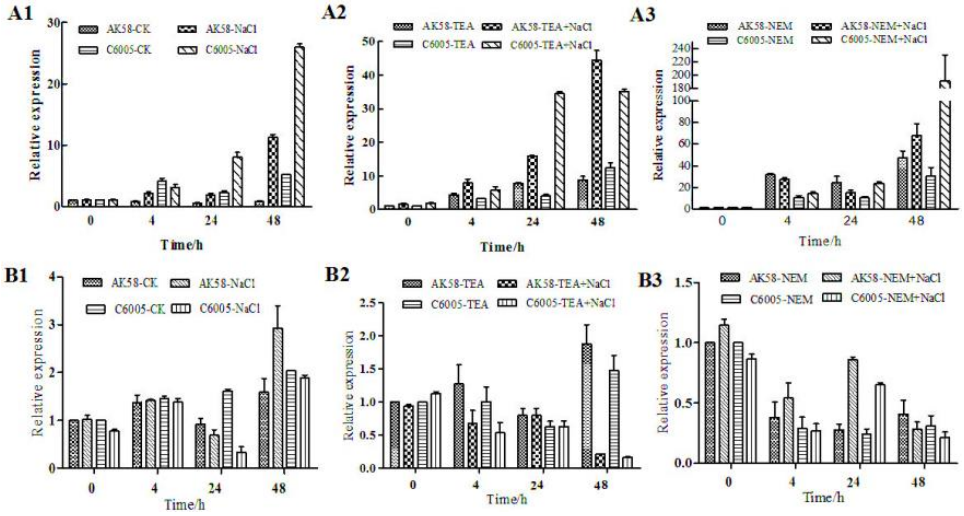

Fig. 6: Effects of NaCl and blockers stress on the expression of K^+ transporters gene *HKA5* (A) and *HKT1* (B) in roots of two wheat cultivars

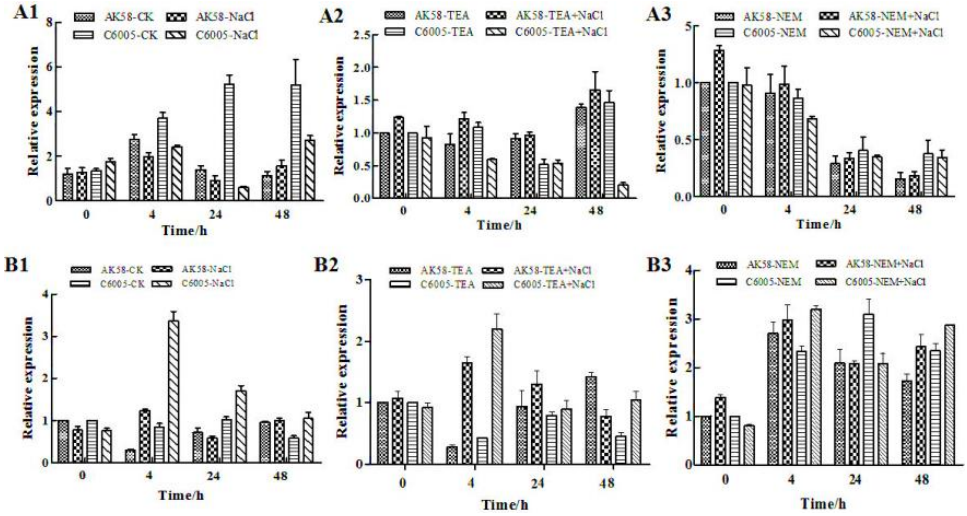

Fig. 7: Effects of blockers and salt stress on the expression of K^+ channels gene *AKT1* (A) and *KAT2* (B) in roots of two wheat cultivars

salt stress than 'C6005'.

NaCl induced plasma membrane depolarization could activate the K^+ outward rectification channels and some NSCCs which mediate K^+ efflux, and inhibit the K^+ inward rectification and other NSCCs which mediate K^+ influx (Demidchik and Maathuis, 2007; Chen *et al*., 2015b). Although K^+ efflux was active, K^+ transporters could still contribute to K^+ uptake in some ways and reduce the net K^+ efflux in generally. Researches on rice knockout mutants and overexpression lines of OsHAK5 showed that OsHAK5 contribute to the K^+ acquisition, mobilization and salt tolerance in shoot growth (Yang *et al*., 2014). The KT/HAK/KUP family in wheat was rarely well studied. K^+ fluxes monitoring by NMT showed a two to six times increase of K^+ efflux in NEM and NaCl combine treated root tips, suggesting that K^+ transporters had been a mainstay on the K^+ uptake under higher Na^+ concentration. The low-affinity K^+ channels, which rely on the membrane hyperpolarization, may be inhibited by the depolarization of plasma membrane. While NSCCs widely existed on the plasma membrane and endomembrane system, may be the important pathways, which mediate the Na^+ into the cytoplasm under NaCl stress (Demidchik and Tester, 2002). In this study, K^+ uptake through NSCCs and K^+ channel under 250 m*M* NaCl stress conditions were observed in 'AK58' and 'C6005', respectively, indicating that the salt-tolerant 'C6005' was less influenced by

depolarization owing to salt shock. K^+ release from roots under NaCl stress was just a result of membrane disintegrity or osmotic shock (Coskun *et al*., 2013), smaller K^+ efflux is related to higher salt tolerance and NaCl-induced K^+ efflux could be a physiological "marker" for predicting the salt-tolerance (Cuin *et al*., 2008).

Plasma membrane H^+-ATPase and H^+-PPase provide the driving force for K^+ uptake through K^+ transporters (Yan *et al*., 2002). The H^+-ATPase activities were inhibited obviously by NaCl treatment, but were less influenced in 'C6005' than in 'AK58', positively correlate to their salt tolerance. NEM treatment induced a significant decline of H^+-ATPase activities in two wheat cultivars with a much more decrease in 'C6005' (82.03%) than in 'AK58' (58.69%), suggesting that 'C6005' was more dependent on the K^+ transporter under salt stress, given a positive correlation exist between the activities of H^+-ATPase and K^+ transporters. Recently a dual localization of H^+-PPase was proved to exist in the vacuole and plasma membrane in *Arabidopsis* (Alexandersson *et al*., 2004). The present study also detected H^+-PPase activity in PM and showed an obvious increase of PM-H^+-PPase activity in 'AK58' under NaCl stress condition, but diametrically opposed trends in 'C6005'. Transgenic *Arabidopsis* plants overexpressing *TaTVP1* showed a better tolerance to high concentrations of NaCl (Faïçal *et al*., 2007).

The K^+/H^+ symport dominated the K^+ transport in high affinity range, but the symport activity could be down regulated by membrane depolarization (Maathuis and Sanders, 1997). Different genotype response of K^+/H^+ transport activities to 250 m*M* NaCl stress was observed, 'C6005' had a higher K^+/H^+ transport activities than 'AK58' under salt tress and was more sensitive to NEM treatment, the K^+/H^+ transport activities of 'C6005' in combined NEM and NaCl treatment decreased by 77.3%, compared to relative single NaCl treatment, while the data for 'AK58' was only 29.1%. These results indicated that salt tolerant-cultivar had relied more on K^+ transporters for K^+ uptake than did the salt-sensitive cultivar under high NaCl concentration condition. However, the confusing contradiction between the fall of H^+-ATPase activities and the rise of K^+/H^+ transporters activities existed in NaCl treated 'C6005' need to be further studied. Compared with K^+ transporters, K^+ channels, which rely on membrane hyperpolarezation should be more influenced by NaCl stress (Nieves-Cordones *et al*., 2014). K^+ channels include K^+ inward channels and K^+ outward channels (Sharma *et al*., 2013). The researches on NaCl stress mainly focus on voltage-dependent K^+ channels, such as shaker-like K^+ channels which include K^+ inward-rectifying channel and K^+ outward-rectifying channel (Dreyer and Uozumi, 2011). 10 m*M* NaCl induced an expected weakened K^+ current in the protoplast of the two wheat cultivars, and the drop rate in 'C6005' (23.56%) was lower than in 'AK58' (41.5%). The K^+ inward-rectifying channels of salt sensitive cultivar 'AK58' were easier to be inhibited by NaCl treatment than did the salt tolerance cultivar 'C6005', suggesting that the K^+ channels in the salt-sensitive cultivar may be more likely to respond to NaCl stress. Extracellular Na^+ shock lead to the depolarization of plasma membrane, then the activation of K^+ out-ward channels and the weakness of K^+ in-ward currents (Zepedajazo *et al*., 2008; Ma *et al*., 2012). The researches on rice showed that the 150 m*M* NaCl stress inducted the suppression of K^+ channels gene *OsAKT1* in the exodermis of salt-tolerance cultivar. Others reported that AKT type K^+ channels in rice may work for decreasing Na^+ uptake instead of K^+ uptake (Golldack *et al*., 2003).

K^+ transporters and channels play a part in the salt tolerance by mediating the K^+ uptake. KT/HAK/KUP family contributed to the high-affinity K^+ uptake, the upregulation of HAK5 benefits salt tolerance when NaCl induced dramatic decline in intracellular K^+ concentration (Grabove, 2007). The TaHAK5 gene was located on chromosome 3DL in wheat genome; however, its function on K^+ uptake had not been proved. The studies showed that 250 m*M* NaCl increased the transcript abundance of TaHAK5 in two wheat cultivars, with significantly higher in 'C6005' than in 'AK58' in 48 h under NaCl stress. TEA and NEM blockers both intensified the up-regulation of TaHAK5 and a surprising approximately 200 fold increase was found in combined NEM and NaCl treatment of 'C6005'. Yang *et al*. (2014) also found that OsHAK5 transcript levels were increased transiently by about 10-fold at 1 h after K^+ removal in rice root, the overexpression of OsHAK5 could be helpful to maintain K^+/Na^+ ratio and salt stress tolerance. Alemán *et al*. (2009) compared the expression of ThHAK5 from halophyte (*Thellungiella halophila*) and AtHAK5 from glycophyte (*Arabidopsis thaliana*) and found that the regulation of HAK5 may differ between glycophyte and halophytes grown under salinity, indicating that plants which differ in salt tolerance show differential regulation of the expression of genes encoding K+ transporters. The research on TaHKT was much wider and deeper than on TaHAK5, Group 1 of HKT genes were considered to transport Na^+ specifically in Arabidopsis, rice and wheat. However, there was no consensus as to whether group 2 of HKT could transport K^+ or Na^+ (Byrt *et al*., 2007; Byrt *et al*., 2014). The present study showed that the transcript level of TaHKT1 was less influenced by NaCl stress or the two K^+ uptake blockers than did TaHAK5. Wang *et al*. (2014) found that 10 m*M* TEA could reduce Na^+ influx by 42% in athkt1;1 mutant and thus AtHKT1 was proved to mediate the low-affinity Na^+ uptake. Zhang *et al*. (2015b) indicated that OsHKT1; 1 was helpful to transport Na^+ to roots in xylem and reduced the damage of NaCl stress. Similar to previous studies, TaHKT1 may be more involved in the Na^+ transport rather than the K^+ uptake. The expression of TaHAK5 was induced by NaCl stress and showed an obvious different genotype response to NaCl, this results were validated each other with the preceding physiology experiments, which exhibited K^+ transporters take more responsibility in salt

tolerance than K^+ channels or NSCCs.

AKT1 are responsible for K^+ uptake as an important Shaker-like potassium channel at the external K^+ concentration above 0.01 m*M* in Arabidopsis (Rubio *et al*., 2010; Caballero *et al*., 2012; Sharma *et al*., 2013). OsAKT1 transcripts were decreased in response to 150 m*M* NaCl or D-sorbitol solution in both root and shoot of rice (Fuchs and Hedrich, 2005). 250 m*M* NaCl could induce a significant down-regulation of TaAKT1 in two wheat roots after 4 h treatment and the down-regulation in 'C6005' was more obvious, the results were similar to the previous studies on Arabidopsis and rice. Fuchs and Hedrich (2005) considered that the down-regulation of OsAKT1 was induced by a general reduction in vigor under osmotic stress. As another Shaker-like potassium channel, KAT family was expressed in *A. thaliana* and rice guard cells (Schachtman and Gaber, 1992; Obata *et al*., 2007). AtKAT1 and AtKAT2 have been proved to be expressed in guard cells to mediate K^+ uptake when stomata open. The transcripts of AtKAT2 were also found in the phloem parenchyma of leaf tissue (Philippar *et al*., 2004). Hwang *et al*. (2013) concluded that OsKAT2 was predominantly expressed in rice guard cells and may be a functional orthologue of the Arabidopsis AtKAT1. We isolated an AtKAT2-like potassium channel (TaKAT2) in wheat, which has a homology with AtKAT2 in Arabidopsis. Although preceding researches have proved that AtKAT2 exits in guard cell and phloem (Xicluna *et al*., 2007), we found an obvious increase in transcript level of TaKAT2 in roots on 250 m*M* NaCl stress for 4 and 24 h. Double expression of TaKAT2 could happen in phloem of wheat roots in response to low K^+ from root epidermis and cortex.

Conclusion

The elevation of transcription level of K^+ transporters was beneficial to K^+ holding capacity of wheat roots. Nevertheless, how other ways of K^+ acquisition and depletion do participate in the transport of K^+ under salt stress needs to be studied by cell and molecular biology experiments.

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References

Ahanger, M.A. and R.M. Agarwal, 2017. Salinity stress induced alterations in antioxidant metabolism and nitrogen assimilation in wheat (*Triticum aestivum* L.) as influenced by potassium supplementation. *Plant Physiol. Biotechnol.*, 115: 449–460

Ahmad, I., A. Mian and F.J. Maathuis, 2016. Overexpression of the rice AKT1 potassium channel affects potassium nutrition and rice drought tolerance. *J. Exp. Bot.*, 67: 2689–2698

Ahn, S.J., M. Sivaguru and H. Osawa, 2001. Aluminum inhibits the H^+-ATPase activity by permanently altering the plasma membrane surface potentials in squash roots. *Plant Physiol.*, 126: 1381–1390

Alexandersson, E., G. Saalbach and C. Larsson, 2004. Arabidopsis plasma membrane proteomics identifies components of transport, signal transduction and membrane trafficking. *Plant Cell Physiol.*, 45: 1543–1556

Alemán, F., M. Nieves-Cordones and V. Martínez, 2009. Differential regulation of the HAK5 genes encoding the high-affinity K^+ transporters of *Thellungiella halophila* and *Arabidopsis thaliana*. *Environ. Exp. Bot.*, 65: 263–269

Babgohari, M.Z., A. Niazi and A.A. Moghadam, 2013. Genome-wide analysis of key salinity-tolerance transporter (HKT1; 5) in wheat and wild wheat relatives (A and D genomes). *In Vitro Cell Dev. Plant*, 49: 97–106

Buschmann, P.H., R. Vaidyanathan and W. Gassmann, 2000. 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: 1387

Brauer, E.K., N. Ahsan and R. Dale, 2016. The Raf-like kinase ILK1 and the high affinity K^+ transporter HAK5 are required for innate immunity and abiotic stress response. *Plant Physiol.*, 171: 1470–1484

Byrt, C.S., J.D. Platten and W. Spielmeyer, 2007. HKT1;5-like cation transporters linked to Na^+ exclusion loci in wheat, NAX2 and KNA1. *Plant Physiol.*, 143: 1918–1928

Byrt, C.S., B. Xu and M. Krishnan, 2014. The Na^+ transporter, *TaHKT1; 5-D*, limits shoot Na^+ accumulation in bread wheat. *Plant J.*, 80: 516–526

Caballero, F., M.A. Botella and L.A. Rubio, 2012. Ca^{2+}-Sensitive system mediates low-affinity K^+ uptake in the absence of AKT1 in Arabidopsis plants. *Plant Cell Physiol.*, 53: 2047

Chen, Z., I.I. Pottosin and T.A. Cuin, 2007. Root plasma membrane transporters controlling K^+/Na^+ homeostasis in salt-stressed barley. *Plant Physiol.*, 145: 1714

Chen, J., W.H. Wang and F.H. Wu, 2015a. Hydrogen sulfide enhances salt tolerance through nitric oxide-mediated maintenance of ion homeostasis in barley seedling roots. *Sci. Rep.*, 5: 12516

Chen, G., Q. Hu and L. Luo, 2015b. Rice potassium transporter OsHAK1 is essential for maintaining potassium-mediated growth and functions in salt tolerance over low and high potassium concentration ranges. *Plant Cell Environ.*, 38: 2747

Coskun, D., D.T. Britto and Y.K. Jean, 2013. K^+ efflux and retention in response to NaCl stress do not predict salt tolerance in contrasting genotypes of rice (*Oryza sativa* L.) *PloS One*, 8: e57767

Cuin, T.A., S.A. Betts and R. Chalmandrier, 2008. A root's ability to retain K^+ correlates with salt tolerance in wheat. *J. Exp. Bot.*, 59: 2697b

Deinlein, U., A.B. Stephan and T. Horie, 2014. Plant salt-tolerance mechanisms. *Trends Plant Sci.*, 19: 371–379

Demidchik, V. and F.J. Maathuis, 2007. Physiological roles of nonselective cation channels in plants: from salt stress to signalling and development. *New Phytol.*, 175: 387–404

Demidchik, V. and M. Tester, 2002. Sodium fluxes through nonselective cation channels in the plasma membrane of protoplasts from Arabidopsis roots. *Plant Physiol.*, 128: 379–387

Ding, T.L., P. Duan and B.S. Wang, 2006. Na^+/K^+ selectivity of leaf sheath in wheat cultivars differing in salt tolerance. *J. Plant Physiol. Mol. Biol.*, 32: 123

Dreyer, I. and N. Uozumi, 2011. Potassium channels in plant cells. *FEBS J.*, 278: 4293–4303

Faïçal, B., M. Hanin and I. Mezghani, 2007. Overexpression of wheat Na^+/H^+ antiporter *TNHX1* and H^+-pyrophosphatase TVP1 improve salt- and drought-stress tolerance in *Arabidopsis thaliana* plants. *J. Exp. Bot.*, 58: 301–308

Fahad, S., S. Hussain and A. Matloob, 2015. Phytohormones and plant responses to salinity stress: A review. *Plant Growth Regul.*, 75: 391–404

Faraday, C.D. and R.M. Spanswick, 1992. Maize root plasma membranes isolated by aqueous polymer two-phase partitioning: Assessment of residual tonoplast ATPase and pyrophosphatase activities. *J. Exp. Bot.*, 43: 1583–1590

Fuchs, I. and R. Hedrich, 2005. Rice K^+ uptake channel *OsAKT1* is sensitive to salt stress. *Planta*, 22: 212–221

Golldack, D., F. Quigley and C.B. Michalowski, 2003. Salinity stress-tolerant and -sensitive rice (*Oryza sativa* L.) regulate AKT1-type potassium channel transcripts differently. *Plant Mol. Biol*., 51: 71–81

Grabove, A., 2007. Plant KT/KUP/HAK potassium transporters: single family-multiple functions. *Ann. Bot.*, 99: 1035–1041

Hwang, H., J. Yoon and H.Y. Kim, 2013. Unique features of two potassium channels, *OsKAT2* and *OsKAT3*, expressed in rice guard cells. *PloS One*, 8: e72541

Jones, C.G., J.D. Hare, S.J. Compton. 1989. Measuring plant protein with the Bradford assay. *J. Chem. Ecol.*, 15: 979-992

Li, H., H. Zhang and G. Li, 2015. Expression of maize heat shock transcription factor gene ZmHsf06 enhances the thermostolerance and drought-stress tolerance of transgenic Arabidopsis. *Funct. Plant Biol*., 42: 1080–1091

Lin, H., W. Du and Y. Yang, 2014. A calcium-independent activation of the Arabidopsis SOS2-like protein kinase24 by its interacting SOS3-like calcium binding protein1. *Plant Physiol.*, 164: 2197–206

Ma, L., H. Zhang and L. Sun, 2012. NADPH oxidase AtrbohD and AtrbohF function in ROS-dependent regulation of Na^+/K^+ homeostasis in Arabidopsis under salt stress. *J. Exp. Bot.*, 63: 305

Maathuis, F.J. and D. Sanders, 1997. Regulation of K^+ absorption in plant root cells by external K^+: interplay of different plasma membrane K^+ transporters. *J. Exp. Bot*., 48: 451

Munns, R. and M. Tester, 2008. Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol*., 59: 651

Nieves-Cordones, M., F. Alemán and V. Martínez, 2014. K^+ uptake in plant roots. The systems involved their regulation and parallels in other organisms. *J. Plant Physiol.*, 171: 688–695

Obata, T., H.K. Kitamoto and A. Nakamura, 2007. Rice shaker potassium channel OsKAT1 confers tolerance to salinity stress on yeast and rice cells. *Plant Physiol*., 144: 1978–1985

Philippar, K., N. Ivashikina and P. Ache, 2004. Auxin activates KAT1 and KAT2, two K^+-channel genes expressed in seedlings of Arabidopsis thaliana. *Plant J*., 37: 815–827

Rubio, F., F. Alemán and M. Nieves-Cordones, 2010. Studies on Arabidopsis athak5, atakt1 double mutants disclose the range of concentrations at which *AtHAK5*, *AtAKT1* and unknown systems mediate K^+ uptake. *Physiol. Plantarum*, 139: 220–228

Rubio, F., M. Fon and R. Ródenas, 2014. A low K^+ signal is required for functional high-affinity K^+ uptake through HAK5 transporters. *Physiol. Plantarum*, 152: 558–570

Schachtman, D.P. and B.R. Terry, 1991. The K/Na selectivity of a cation channel in the plasma membrane of root cells does not differ in salt-tolerant and salt-sensitive wheat species. *Plant Physiol.*, 97: 598–605

Schachtman, D.P. and R.F. Gaber, 1992. Expression of an inward-rectifying potassium channel by the Arabidopsis KAT1 cDNA. *Science*, 258: 1654

Shabala, S. and T.A. Cuin, 2008. Potassium transport and plant salt tolerance. *Physiol. Plantarum*, 133: 651

Shabala, S. and I. Pottosin, 2014. Regulation of potassium transport in plants under hostile conditions: implications for abiotic and biotic stress tolerance. *Physiol. Plantarum*, 151: 257–279

Sharma, T., I. Dreyer and J. Riedelsberger, 2013. The role of K^+ channels in uptake and redistribution of potassium in the model plant Arabidopsis thaliana. *Front Plant Sci*., 4: 224

Shen, Y., L. Shen and Z. Shen, 2016. The potassium transporter *OsHAK21* functions in the maintenance of ion homeostasis and tolerance to salt stress in rice. *Plant Cell Environ.*, 38: 2766–2779

Shen, H., J. Chen and Z. Wang, 2006. Root plasma membrane H^+-ATPase is involved in the adaptation of soybean to phosphorus starvation. *J. Exp. Bot.*, 57: 1353

Vera-Estrella, R., B.J. Barkla and L. García-Ramírez, 2005. Salt stress in *Thellungiella halophila* activates Na^+ transport mechanisms required for salinity tolerance. *Plant Physiol.*, 139: 1507–1517

Wang, Y. and W.H. Wu, 2013. Potassium transport and signaling in higher plants. *Annu. Rev. Plant Biol.*, 64: 451

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

Widodo, J., H. Patterson and E. Newbigin, 2009. Metabolic responses to salt stress of barley (*Hordeum vulgare* L.) cultivars, Sahara and Clipper, which differ in salinity tolerance. *J. Exp. Bot.*, 60: 4089

Xicluna, J., B. Lacombe and I. Dreyer, 2007. Increased functional diversity of plant K^+ channels by preferential heteromerization of the shaker-like subunits AKT2 and KAT2. *J. Biol. Chem.*, 282: 486–494

Yan, F., Y. Zhu and C. Müller, 2002. Adaptation of H^+-pumping and plasma membrane H^+-ATPase activity in proteoid roots of white lupin under phosphate deficiency. *Plant Physiol*., 129: 50–63

Yang, T., S. Zhang and Y. Hu, 2014. The role of a potassium transporter *OsHAK5* in potassium acquisition and transport from roots to shoots in rice at low potassium supply levels. *Plant Physiol.*, 166: 945–959

Yao, F.Y., G.N. Qi and H.M. Ren, 2017. S-type anion channel SLAC1's homologues inhibit inward potassium channels AKT2 and KAT2 in Arabidopsis. *Sci. Bull.*, 62: 464–466

Yu, Y., T. Xu and X. Li, 2016. NaCl-induced changes of ion homeostasis and nitrogen metabolism in two sweet potatoes (*Ipomoea batatas* L.) cultivars exhibit different salt tolerance at adventitious root stage. *Environ. Exp. Bot.*, 129: 23–36

Zhang, Y.M., H.M. Zhang and Z.H. Liu, 2015a. The wheat NHX antiporter gene *TaNHX2* confers salt tolerance in transgenic alfalfa by increasing the retention capacity of intracellular potassium. *Plant Mol. Biol*., 87: 317–327

Zhang, W., R. Wang and W. Jing, 2015b. The *OsHKT1;1* Transporter is involved in salt tolerance and regulated by an MYB-Type Transcription Factor. *Plant Physiol.*, 168: 1076–1090

Zepedajazo, I., S. Shabala and Z. Chen, 2008. Na-K transport in roots under salt stress. *Plant Signal. Behav*., 3: 401–403

Zheng, Y., Z. Wang and X. Sun, 2008. Higher salinity tolerance cultivars of winter wheat relieved senescence at reproductive stage. *Environ. Exp. Bot.*, 62: 129–138

Zhu, J., 2003. Regulation of ion homeostasis under salt stress. *Curr. Opin. Plant Biol.*, 6: 441–445

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