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PtAKT1 maintains selective absorption capacity for K$^+$ over Na$^+$ in halophyte *Puccinellia tenuiflora* under salt stress

Pei Wang[1] · Qiang Guo[1] · Qian Wang[1] · Xiang-Rui Zhou[1] · Suo-Min Wang[1]

Abstract The halophyte *Puccinellia tenuiflora* efficiently restricts Na$^+$ entry into roots and maintains a strong selective absorption (SA) capacity for K$^+$ over Na$^+$ to cope with salt stress. AKT1-type channel, an important plant K$^+$ uptake protein, may be active in regulating SA in *P. tenuiflora*. Here, its encoding gene *PtAKT1* was isolated from *P. tenuiflora*. To examine the function of PtAKT1 in salt resistance and selectivity for K$^+$ over Na$^+$, we analyzed the transcript patterns of *PtAKT1* and Na$^+$, K$^+$ concentration in *P. tenuiflora* during varying concentrations of NaCl and KCl. Results displayed that both the transcription abundance of *PtAKT1* in roots and the whole plant K$^+$ concentration did not change with varying K$^+$ concentrations in the medium (0–10 mM). When NaCl (25 or 150 mM) was present, transcript levels of *PtAKT1* were down-regulated, accompanied by a decrease in the whole plant K$^+$ concentration. However, under normal (5 mM) or low (0.1 mM) KCl condition, transcript levels of *PtAKT1* in roots under severe salt stress (150 mM NaCl) were higher than that in mild salt condition (25 mM NaCl) within 6–24 h, so did SA during the whole treatment time (6–96 h). A strong positive correlation was existed

between root *PtAKT1* transcription abundance and SA values during 25 or 150 mM NaCl plus KCl. Consequently, it is proposed that PtAKT1 is a key factor in regulating selective absorption capacity for K$^+$ over Na$^+$ in *P. tenuiflora* during saline condition. Finally, we hypothesize a model that SOS1, HKT and SKOR synergistically regulate potassium and sodium transport systems in the xylem parenchyma, and subsequently modulates K$^+$ absorption via AKT1, which is located in epidermis, cortex and endodermis. The model could also provide a likely evidence to the mechanism that SOS1 could protect AKT1 mediating K$^+$ influx during salt stress.

Introduction

Salinity is a main abiotic factor limiting crop production worldwide (Flowers 2004). Excessive accumulation of Na$^+$ in plants can damage cell membrane and inhibit enzyme activity of metabolism. Owing to the similar ionic hydrated radius between Na$^+$ and K$^+$, Na$^+$ always compete the binding site of K$^+$ channels and transporters, thus restricts K$^+$ acquisition and induces imbalance of cytoplasmic cation, affecting many physiological and metabolic activities accordingly, for instance, enzyme system homeostasis and osmatic adjustment (Ding and Zhu 1997; Buschmann et al. 2000; Shabala and Cuin 2008; Horie et al. 2009). Maintaining a high K$^+$/Na$^+$ selectivity is important in most plants.

Puccinellia tenuiflora is a typical gramineous halophyte, which is broadly distributed in saline swamplands, with significant feeding and ecological values (Wang et al. 2009; Guo et al. 2012). Our previous researches proved

✉ Suo-Min Wang
  smwang@lzu.edu.cn

[1] State Key Laboratory of Grassland Agro-ecosystems, College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou 730020, People's Republic of China

that limiting Na^+ flow into roots and possessing a powerful selectivity for K^+ contributed to the salt resistance of *P. tenuiflora* (Wang et al. 2009; Guo et al. 2012). Recent research found that plasma membrane Na^+/H^+ antiporter PtSOS1 was a key factor in selective transport capacity for K^+ over Na^+ in *P. tenuiflora* in salt condition (Guo et al. 2012). However, the molecular mechanism of selective absorption (SA) capacity for K^+ over Na^+ in *P. tenuiflora* remains unclear. The research by Peng et al. (2004) demonstrated the ability of *P. tenuiflora* to absorb K^+ under salt stress depended on K^+ channels that inhibited by Cs^+. A further study by Ardie et al. (2010) found that over-expression of *PtAKT1* led to the rise of K^+ content and decrease in Na^+ accumulation in *Arabidopsis thaliana*. These researches indicate that AKT1-type channel is closely related to K^+ uptake in roots and salt tolerance in *P. tenuiflora*.

AKT1 (ARABIDOPSIS K^+ TRANSPORTER1), a Shaker family K^+ channel, is crucial in plant potassium nutrition acquisition. In *A. thaliana*, most of the potassium absorbs through AKT1 and HAK5 (Hirsch et al. 1998; Gierth et al. 2005; Pyo et al. 2010). HAK5 accounts for a large portion of the high-affinity K^+ absorption, and its activity is suppressed by NH_4^+ (Santa-Maria et al. 1997). AKT1 is a NH_4^+-insensitive K^+ uptake component, and mediates K^+ absorption covering a large range of exogenous K^+ concentrations, even at micromolar concentrations (Rubio et al. 2008). The gene encoding AKT1 was the first cloned plant inward-rectifying K^+ channel gene from *A. thaliana* (Sentenac et al. 1992). *AKT1* was located in epidermis, cortex, and endodermis of the root (Cao et al. 1995; Lagarde et al. 1996; Fuchs et al. 2005). In *A. thaliana*, AKT1 was activated by low K^+ medium, but its transcript levels were not influenced by disparate adscititious K^+ from the concentrations range of low to high, implying that the expression of AKT1 was possibly post-transcriptional regulation (Maathuis and Sanders 1996; Maathuis et al. 2003; Spalding et al. 1999; Hampton et al. 2004). In spite of the transcription abundance of *OsAKT1* was not affected by external K^+, *OsAKT1* was down-regulated in *Oryza sativa* responding to salt treatment, in-line with the significant reduction of inward K^+ currents in root protoplasts (Fuchs et al. 2005). Both transcripts and protein expression of AKT1-type K^+ channel McAKT1 in ice plant (*Mesembryanthemum crystallinum*) were also enormously declined under saline condition (Su et al. 2001). These results indicated that AKT1 was inhibited by salt stress. However, AKT1 has been shown to possibly mediate Na^+ influx. In a comparison of two rice varieties with different salt resistance, Golldack et al. (2003) showed higher *OsAKT1* transcript levels in the sensitive cultivar IR29 than that in the tolerant cultivar Pokkali in response to

Na^+. Another research in rice showed that Na^+ in leaf protoplasts of the salt-sensitive cultivar was decreased by approximately 50 % when the K^+ channels inhibitors Cs^+ and TEA^+ were added, whereas the salt-tolerant variety was not influenced by Cs^+ and TEA^+ (Kader and Lindberg 2005). A recent study suggested that AtAKT1 is possibly a channel for K^+ efflux from the root during salt stress (Nieves-Cordones et al. 2010). These results provided entirely different viewpoints about AKT1 under salt stress. Therefore, the role of AKT1 in *P. tenuiflora* needs to be further revealed.

To address the role of AKT1 in salt tolerance, we first isolated and characterized *PtAKT1* gene from *P. tenuiflora*. Second, we investigated gene expression patterns and ion concentrations of potassium and sodium in *P. tenuiflora* under different KCl and NaCl treatments. The results suggested the important role of AKT1 in selective absorption for K^+ over Na^+ in *P. tenuiflora*.

Materials and methods

Plant culture and treatments

Seeds' germination and seedlings' growth conditions of *P. tenuiflora* were performed according to the methods in Wang et al. (2009). One-month-old seedlings were transferred to the modified Hoagland nutrient solution deprived of KNO_3 for 7 days and 5 mM KNO_3 was substituted by 2.5 mM NH_4NO_3. Then the plants were treated as follows: (i) 0, 0.1, 0.5, 1, 5 or 10 mM KCl was added separately for 48 h; (ii) 0.1 or 5 mM KCl plus 25 or 150 mM NaCl for 0, 6, 12, 24, 48, 72 and 96 h, respectively.

Cloning of *PtAKT1* and sequence analysis

Total RNA was isolated from roots under 150 mM NaCl for 48 h using the Trizol Kit (Sangon, China) (Wu et al. 2011; Yuan et al. 2015). Reverse transcription was performed using MMLV-RTase (Sangon, China). A segment of *PtAKT1* was amplified with primers P1 and P2 (Table S1). PCR fragment was ligated to the pUCm-T vector (Sangon, China) after purification and then sequenced by Sangon. The 5′- and 3′-cDNA were acquired using the kit of RNA ligase mediated rapid amplification of 5′- and 3′-cDNA Ends (RLM-RACE, Invitrogen, USA) according to the manufacturer's instructions, 5′- and 3′-ends of *PtAKT1* were amplified using the special primers P3, P4, P5 and P6, respectively (Table S1). The full-length of the *PtAKT1* cDNA was obtained by assembling these fragments. The final sequence was analyzed as described by Guo et al. (2012).

Semi-quantitative RT-PCR

Primers P7 and P8 (Table S1) were used to test the transcript level of PtAKT1. *PtACTIN* (GenBank accession No. FJ545641) was used as the reference gene for RT-PCR experiments. Specific primers of *PtACTIN* t were A1 and A2 (Table S1). The PCR procedure was given below: 2 min at 94 °C; 30 cycles of 30 s at 94 °C, 25 s at 52 °C (*PtAKT1*)/50 s at 56 °C (*PtACTIN*), 40 s at 72 °C. PCR products were separated on 1.0 % agarose gels.

Determination of Na$^+$ and K$^+$ concentration

Roots of *P. tenuiflora* were soaked for 8 min in icy calcium chloride to swap the apoplastic ion and shoots were washed by distilled water to remove surface salts (Wang et al. 2007). Each plant was cut into roots and shoots before they dried off. Put dried samples into 100 mM glacial acetic acid and bath over 90 °C for 2 h to extract cation. Na$^+$ and K$^+$ concentrations were determined with a flame spectrophotometer (Model 410, Sherwood Scientific Ltd, UK).

Calculation of SA value and statistical analysis

Selective absorption (SA) capacity for K$^+$ over Na$^+$ was calculated in accordance with the equation given below: SA = (K$^+$/Na$^+$ in whole plant)/(K$^+$/Na$^+$ in medium) (Wang et al. 2002, 2004). Statistical analysis was performed according to Guo et al. (2012).

Results

Molecular cloning and primary structure of PtAKT1

The full-length cDNA of *PtAKT1* was reconstituted from *P. tenuiflora* by PCR, 5′- and 3′-RACE, it was 3100 bp long with a 5′-UTR of 74 bp nucleotides, a predicted ORF of 2688 bp nucleotides, and a 3′-UTR of 338 bp. The deduced polypeptide was 895 amino acids long with an estimated molecular mass of 100.8 kDa and a theoretical isoelectric point of 8. 3.

Hydrophobicity analysis of the deduced polypeptide showed that PtAKT1 had 6 predicted trans-membrane segments (TM 1–TM 6), a putative cyclic nucleotide binding domain (cNBD) from amino acids residues 409–498, ankyrin (ANK) repeat sequences from residues 572–700, and the pore domain (262–285) that included the characteristic GYGD/E-motif in K$^+$ channels (Fig. 1). Multiple sequence alignment showed that there was high identity between PtAKT1 and known-plant AKT1-type channels, especially HvAKT1 or TaAKT1 (88 %). Phylogenetic analysis of plant AKT1-type K$^+$ channels indicated that the family was divided into two branches, and PtAKT1 had closer relationships with monocotyledons (Fig. 2). Therefore, the results showed that *PtAKT1* isolated from *P. tenuiflora* encoded an AKT1-type K$^+$ channel.

Expression of *PtAKT1* and whole plant K$^+$ accumulation in *P. tenuiflora* treated with KCl

The expression levels of *PtAKT1* were great higher in roots than that in shoots (Fig. 3a, b). However, no significant difference was found in the transcript abundance of *PtAKT1* among various concentrations of external KCl (0–10 mM) for 48 h both in roots and shoots (Fig. 3b). The results suggested that *PtAKT1* was preferentially expressed in the root tissue and its expression level was not affected by external K$^+$ concentrations. Furthermore, additional KCl (0.1–10) had no impact on whole plant K$^+$ accumulation as well (Fig. 3c).

Expression of *PtAKT1* in *P. tenuiflora* under 25 or 150 mM NaCl plus 0.1 or 5 mM KCl treatments

The tissue-specific expression analysis showed that the transcripts of *PtAKT1* were detected primarily in roots under salt treatment (data not shown). With prolonging of salt stress, the transcript abundance of *PtAKT1* was down-regulated sharply and then tended to stabilization after 6 h under 25 mM NaCl, whereas it was down-regulated gradually and tended to stabilization after 24 h under 150 mM NaCl (Fig. 4a, b). In addition, the expression of *PtAKT1* at 150 mM NaCl plus various KCl was significantly higher than that at 25 mM NaCl plus various KCl during 48 h of salt stress, but there were no significant variations among the treatments from 48 to 96 h (Fig. 4b). The results indicated that the transcription abundance of *PtAKT1* was restrained rapidly by mild salt treatment (25 mM NaCl), while it was inhibited slowly by severe salt stress (150 mM NaCl).

The whole plant Na$^+$, K$^+$ accumulation and the relationship between SA values and expression levels of *PtAKT1* in *P. tenuiflora* under 25 or 150 mM NaCl plus 0.1 or 5 mM KCl treatments

The addition of 25 or 150 mM NaCl plus 0.1 or 5 mM KCl all significantly increased whole plant Na$^+$ accumulation with prolonging of the treatments, while the magnitude of the former (25 mM NaCl plus various KCl) was remarkably lower than that of the latter (150 mM NaCl plus various KCl), and the Na$^+$ concentration treated with 150 mM NaCl plus 0.1 mM KCl was higher than that under 150 mM NaCl plus 5 mM KCl (Fig. 5a). Whole
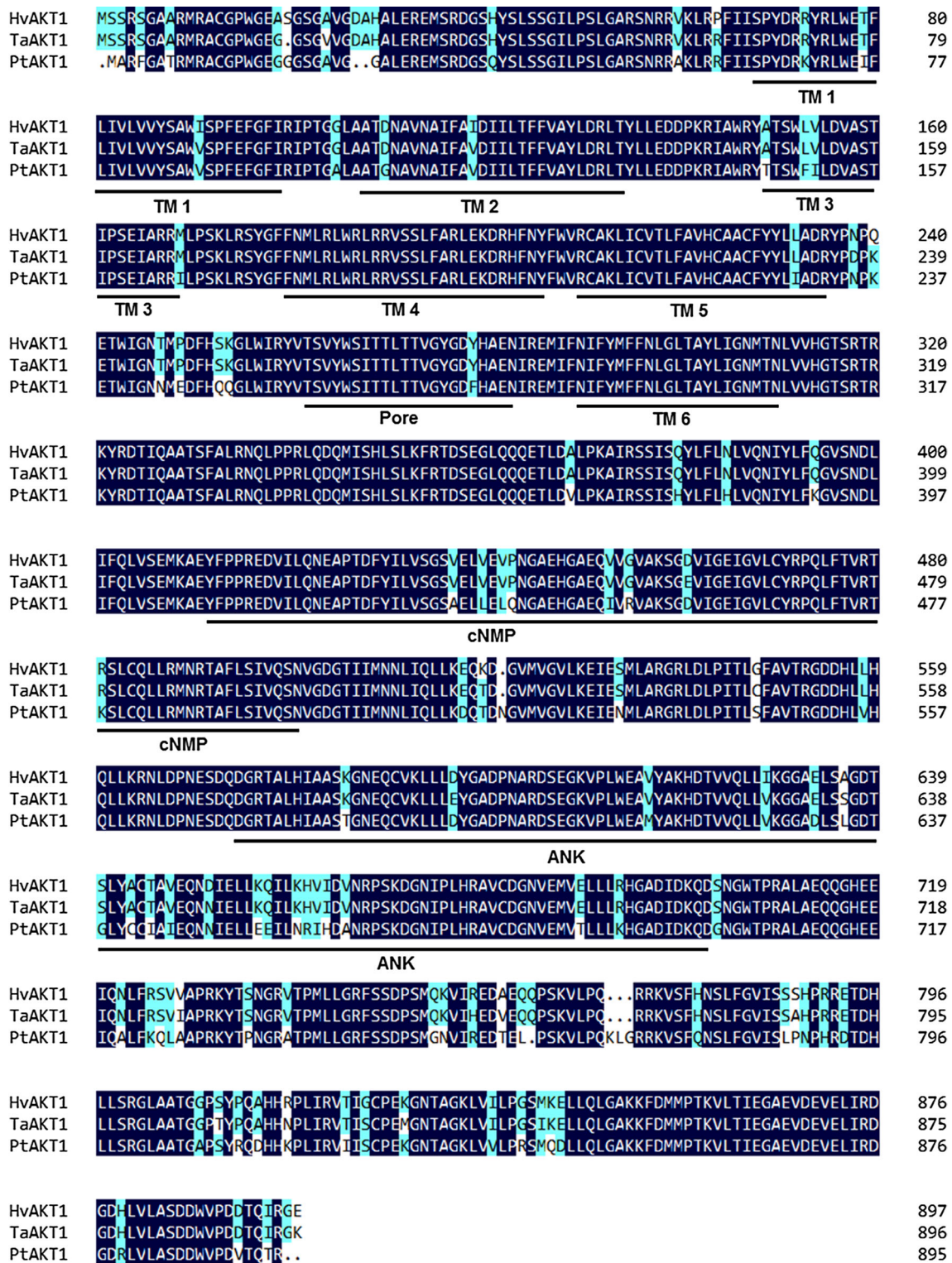
Fig. 1 Sequence alignment of PtAKT1 with other AKT1 from higher plants. Sources of AKT1 and their GenBank accession numbers are as follows: HvAKT1 (*Hordeum vulgare*, DQ465992) and TaAKT1 (*Triticum aestivum*, AF207745). The sequences were aligned with DNAMAN 6.0 software. The six putative trans-membrane domains (TM 1–TM 6) and other domains (pore, cNMP binding domain, and ankyrin repeats domain) are *underlined*, respectively
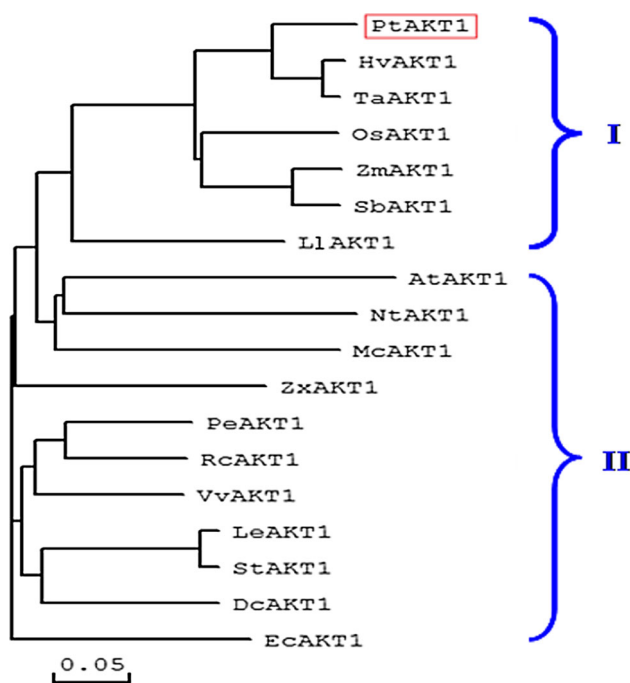
Fig. 2 Phylogenetic tree of PtAKT1 (framed with *red line*) with related sequences in plants. The tree was constructed by the neighbor-joining method. The proteins are as follows: HvAKT1 (*Hordeum vulgare*, DQ465992), TaAKT1 (*Triticum aestivum*, AF207745), OsAKT1 (*Oryza sativa*, AY065970), ZmAKT1 (*Zea mays*, NM_001112010), SbAKT1 (*Sorghum bicolor*, XM002458189), LlAKT1 (*Lilium longiflorum*, EF397611), AtAKT1 (*Arabidopsis thaliana*, AK317729), NtAKT1 (*Nicotiana tabacum*, AB196790), McAKT1 (*Mesembryanthemum crystallinum*, AF267753), ZxAKT1 (*Zygophyllum xanthoxylum*, GQ857474), PeAKT1 (*Populus euphratica*, EU554630), RcAKT1 (*Ricinus communis*, XM_002529327), VvAKT1 (*Vitis vinifera*, FR669116), LeAKT1 (*Lycopersicon esculentum*, X96390), StAKT1 (*Solanum tuberosum*, X86021), DcAKT1 (*Daucus carota*, AJ697979) and EcAKT1 (*Eucalyptus camaldulensis*, AF197332)

plant K^+ accumulation tended to decrease with the increase of stress duration, but no significant difference in the whole plant K^+ accumulation was found among various treatments during 48–96 h (Fig. 5b).

Moreover, selective absorption (SA) capacity for K^+ over Na^+ in roots were on a declining curve under all treatments. Either under high or low KCl conditions, SA values under 150 mM NaCl were significantly greater than that under 25 mM NaCl (Fig. 6a, b). Further analysis showed a significantly positive correlation between SA values and expression levels of *PtAKT1* in roots under 25 or 150 mM NaCl plus various KCl in all treatment times (Fig. 6c, d).

Discussion

The deduced polypeptide sequence of PtAKT1 in the current study showed the typical features that included six trans-membrane segments (TM 1–TM 6) and the pore
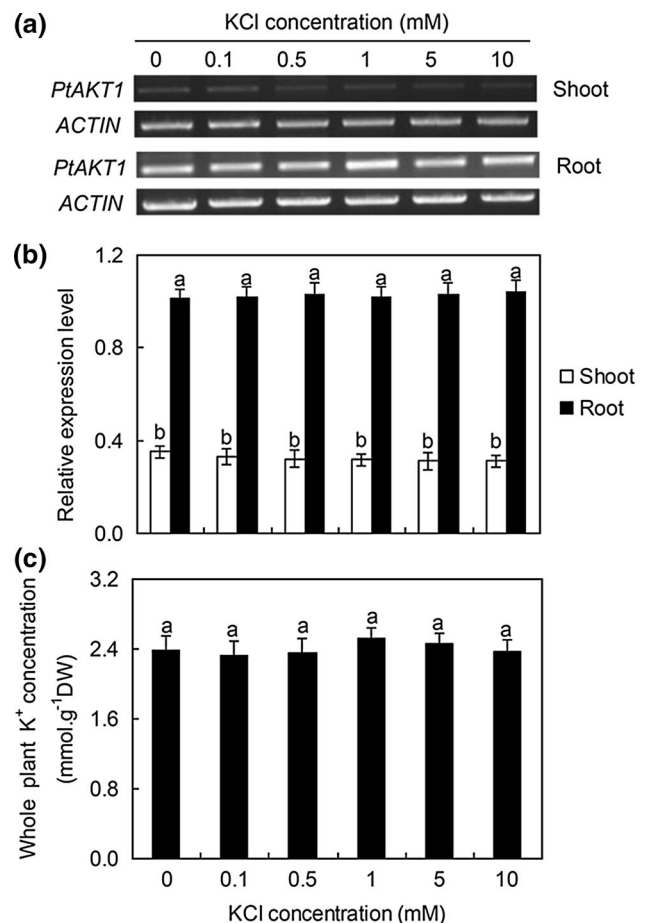

Fig. 3 Expression of *PtAKT1* and whole plant K^+ concentration in *P. tenuiflora* under different concentrations of KCl (0, 0.1, 0.5, 1, 5 and 10 mM) for 48 h. **a** Semi-quantitative RT-PCR analysis of *PtAKT1* in 4-week-old plants exposed to K^+-free nutrient solution for 7 days and then treated with KCl (0, 0.1, 0.5, 1, 5 and 10 mM) for 48 h. **b** The relative expression level of *PtAKT1* (related to *ACTIN*) in shoots and roots. *ACTIN* was used as an internal control. Experiments were repeated at least three times to obtain similar results. **c** Whole plant K^+ concentration in *P. tenuiflora* under different concentrations of KCl for 48 h. Ten plants were pooled in each replicate ($n = 8$). Values are mean ± SE and *bars* indicate SE ($n = 3$–8). *Different letters* indicate significant difference at $P < 0.05$ (Duncan's test)

domain between TM 5 and TM 6, the TM 4 is supposed to be a voltage sensor (Chérel 2004). Similar to AKT1 in *A. thaliana* (Lee et al. 2007), OsAKT1 in *O. sativa* (Fuchs et al. 2005), TaAKT1 in wheat (Buschmann et al. 2000), and McAKT1 in *M. crystallinum* (Su et al. 2001), PtAKT1 possesses a pore domain between TM 5–TM 6 that included the characteristic GYGD/E-motif (Chérel 2004, Fig. 1). The long C-terminal region contained a cyclic nucleotide binding domain (CNBD) and ankyrin repeats, which are thought to be potentially involved in interacting between membrane proteins and cytoskeleton (Sentenac et al. 1992; Véry and Sentenac 2003). Furthermore, the PtAKT1 showed 88 % identity with the HvAKT1 from *H.*
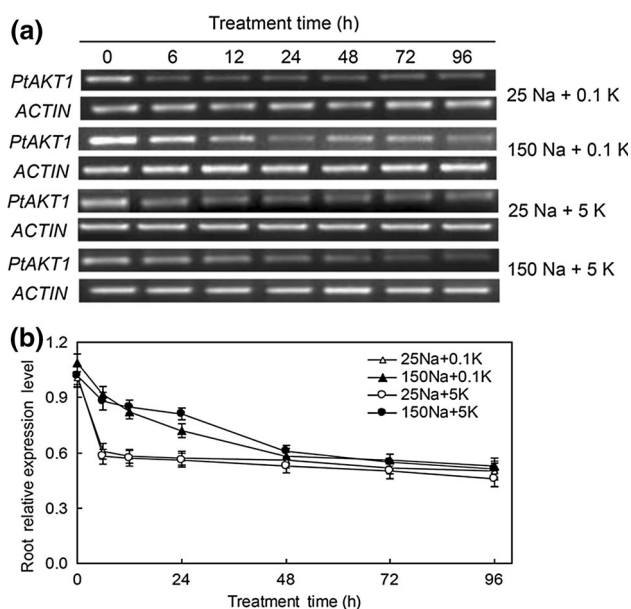
Fig. 4 Time courses of *PtAKT1* expression in roots of *P. tenuiflora* under 25 or 150 mM NaCl plus 0.1 or 5 mM KCl. **a** Semi-quantitative RT-PCR analysis of *PtAKT1* in 4-week-old plants exposed to K^+-free nutrient solution for 7 days and then treated with 25 or 150 mM NaCl plus 0.1 or 5 mM KCl over a 96-h period. **b** The relative expression level of *PtAKT1* (related to *ACTIN*) in roots. *ACTIN* was used as an internal control. Experiments were repeated at least three times to obtain similar results. Values are mean ± SE ($n = 3$) and *bars* indicate SE

vulgare in amino acid sequence (Fig. 1). Phylogenetic analysis showed that PtAKT1 shared a closer relationship with monocotyledon AKT1-type K^+ channels (Fig. 2). These results showed that *PtAKT1* encoded an AKT1-type channel in *P. tenuiflora*.

In line with previous results from *A. thaliana* (Cao et al. 1995) and rice (Fuchs et al. 2005), we reported here that *PtAKT1* expressed primarily in roots of *P. tenuiflora* (Fig. 3a, b). In *A. thaliana*, the steady-state level of *AKT1* mRNA was not influenced by the concentrations changing of additive K^+ (5 μM–5 mM) (Lagarde et al. 1996). The similar results were acquired with Northern blot and micro array (Maathuis and Sanders 1996; Maathuis et al. 2003;

Spalding et al. 1999; Hampton et al. 2004). In *P. tenuiflora*, the transcript level of *PtAKT1* was also unaffected by varying K^+ concentration in the nutrient solution (Fig. 3b), suggesting that the regulation of external K^+ to PtAKT1 could be a post-transcriptional process. Ardie et al. (2010) showed *PtAKT1* was induced by K^+ starvation, while external KCl was added after the treatment of depriving of K^+ in the current study, therefore, our results did not conflict with the finding by Ardie et al. (2010). Furthermore, the whole plant K^+ concentration remained unchanged with varying K^+ in the medium (Fig. 3c), suggesting K^+ uptake in *P. tenuiflora* covered a large range of external K^+ concentrations. It is well known that AKT1 mediates both high- and low-affinity K^+ uptake (Hirsch et al. 1998; Rubio et al. 2008). Therefore, PtAKT1 might be the leading component of K^+ uptake in *P. tenuiflora*, especially with existence of NH_4^+.

In our current study, the expression abundances of *PtAKT1* were down-regulated by NaCl, either under normal (5 mM) or low (0.1 mM) K^+ concentrations (Fig. 4a, b). Similar expression patterns in *AKT1* were noted in *M. crystallinum* (Su et al. 2001), rice (Fuchs et al. 2005), *A. thaliana* (Kaddour et al. 2009) and barley (Boscari et al. 2009). In addition, the whole plant K^+ concentrations showed a decrease trend during salt treatment (Fig. 5b). Similar results were found in rice using electrophysiological approach that the *OsAKT1* in roots was down-regulated under salt treatment, together with significant reduction in the inward K^+ currents (Fuchs et al. 2005). NaCl can depolarize the membrane potential, which results in K^+ absorption via the K^+ inward-rectifying channel is blocked (Shabala and Cuin 2008; Nieves-Cordones et al. 2010). It appeared that the down-regulation of AKT1 gene expression was likely to be attributed to Na^+ entry to cytoplasm. In contrast, Ardie et al. (2010) showed external NaCl (300 mM) did not affect the transcript level of *PtAKT1*, differences in the results possibly due to different Na^+ treatment conditions, on the one hand, 300 mM NaCl is likely to exceed the tolerant range of *P. tenuiflora*; on the other hand, addition of NaCl is on the basis of K^+

Fig. 5 Time courses of whole plant Na^+ (**a**) and K^+ (**b**) concentration in *P. tenuiflora* under 25 or 150 mM NaCl plus 0.1 or 5 mM KCl over a 96-h period. Ten plants were pooled in each replicate ($n = 8$). Values are mean ± SE ($n = 8$) and *bars* indicate SE
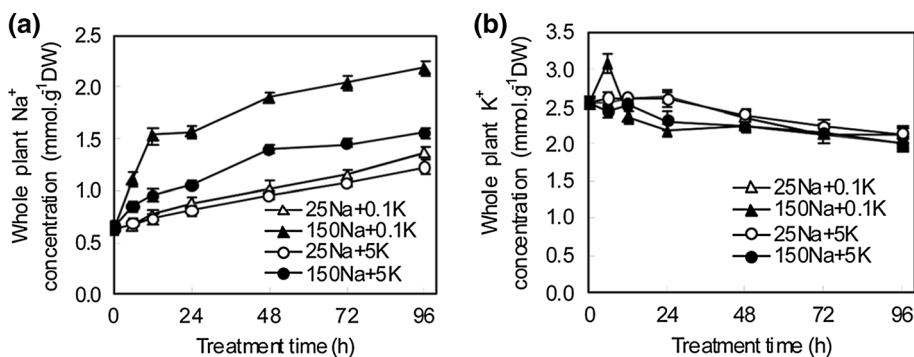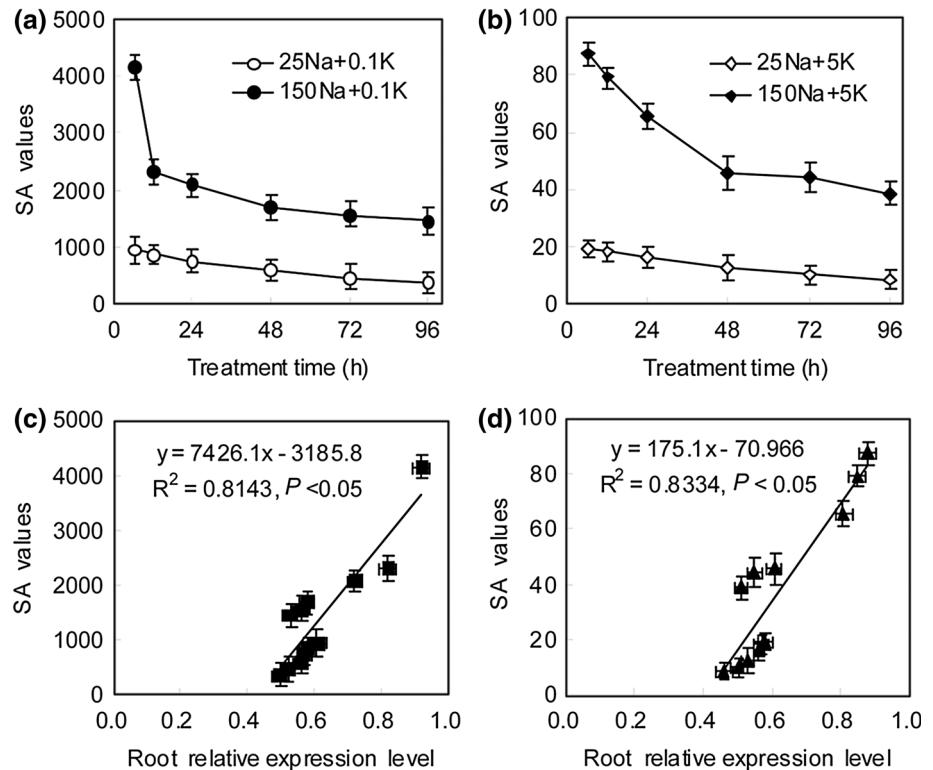
Fig. 6 Selective absorption (SA) capacity for K^+ over Na^+ in *P. tenuiflora* exposed to 25 or 150 mM NaCl plus 0.1 (**a**) or 5 mM KCl (**b**) and relationship between SA values and relative *PtAKT1* expression levels in roots under 25 or 150 mM NaCl plus 0.1 (**c**) or 5 mM KCl (**d**) treatments for 6–96 h. Ten plants were pooled in each replicate ($n = 8$). Values are mean ± SE ($n = 3$–8) and *bars* indicate SE

starvation in our study, similarly, treatment with NaCl inhibited the expression of *PtAKT1* that induced by K^+ starvation in the previous research (Ardie et al. 2010).

Regulating plant K^+ acquisition and maintaining the homeostasis of K^+ and Na^+ in protoplasts are especially important for plants to cope with salt stress. Wang et al. (2009) showed that limiting Na^+ flow into *P. tenuiflora* roots and maintaining a powerful selectivity for K^+ over Na^+ were crucial to salt resistance of the plants. An augment in K^+ content and a reduction in Na^+ accumulation in transgenic plants of *A. thaliana* over-expressed with *PtAKT1* provided a candidate who controls selective absorption (SA) capacity for K^+ over Na^+ in molecular aspect (Ardie et al. 2010). In the present study, although salt treatment inhibited the expression of *PtAKT1*, the transcript levels of *PtAKT1* under severe salt treatment (150 mM NaCl) were dramatically higher than that under mild salt condition (25 mM NaCl) within 6–24 h (Fig. 4b). Similarly, either under normal (5 mM) or low (0.1 mM) KCl condition, the SA values in roots during 150 mM NaCl were significantly higher than that during 25 mM NaCl treatments (Fig. 6a, b). A significant positive correlation was found between root *PtAKT1* expression levels and SA values under either 25 or 150 mM NaCl plus KCl treatments (Fig. 6c, d), indicating that PtAKT1 is very likely to be the key factor in maintaining selective absorption capacity for K^+ over Na^+ during saline conditions.

AKT1-type K^+ channels exhibit a high selectivity for K^+ (Bertl et al. 1997). Over-expression of *PtAKT1* results in not only an augment in K^+ content but also reduction in Na^+ accumulation in *A. thaliana* (Ardie et al. 2010). An interesting study by Qi and Spalding (2004) proposed that AKT1 K^+-acquisition system was protected by SOS1 (plasma membrane Na^+/H^+ antiporter) system from impairment by Na^+, and SOS1 mutation inhibited K^+ absorption in roots under salt stress. In fact, as a Na^+ efflux transporter, SOS1 was deemed to be related to K^+ nutrition in the original researches: Wu et al. (1996) and Ding and Zhu (1997) showed the defect of high-affinity K^+ uptake in *sos1* mutant. Meanwhile, SOS system may restrain Na^+ transporter HKT (high-affinity K^+ transporter) (Rus et al. 2001; Uozumi et al. 2000; Fuchs et al. 2005). Recently, we hypothesized a function model of SOS1 and HKT in sustaining the membrane integrity to regulate K^+ and Na^+ transport system (Guo et al. 2012). In the current work, we suggested that PtAKT1 regulated selective absorption capacity for K^+ over Na^+. However, the transcript levels of *PtAKT1* were down-regulated by NaCl, and the phenomenon that the expression of *PtAKT1* under severe salt condition were higher than that under mild salinity seemed to be difficult to explain. Based on all these results, we supplement the previous model (Guo et al. 2012) in this study and suggest a model to detail the role of AKT1 in maintaining selective absorption capacity for K^+ over Na^+ by roots (Fig. 7). During severe salt stress (Fig. 7a), Na^+ in
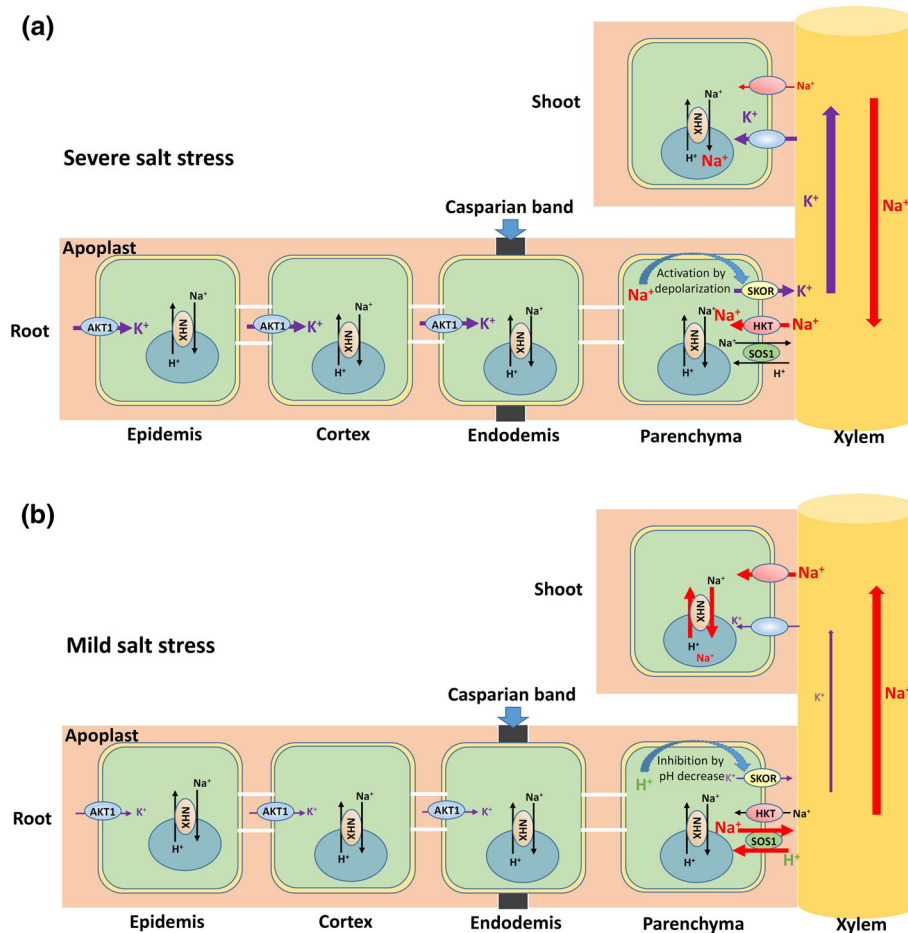
Fig. 7 Schematic model for the function of AKT1 in maintaining selective absorption capacity for K^+ over Na^+ under various salt stress. **a** Under severe salt stress, the transport activities of HKT outweigh SOS1 at the membrane of XPCs and therefore, Na^+ is retrieved from xylem (Guo et al. 2012), Na^+ unloading into XPCs possibly depolarises their plasma membrane, which in turn could activate SKOR to load K^+ into xylem (Wegner and De Boer 1997; Gaymard et al. 1998; Horie et al. 2009), then stimulates AKT1 to absorb and transport more K^+ by feedback regulation. **b** Under mild

salt condition, the activities of SOS1 overwhelm HKT (Guo et al. 2012), SOS1 loads Na^+ to xylem directly and transfer it to shoots, at the same time, hydrion is pumped into XPCs by this Na^+/H^+ antiporter, causing decline in pH of XPCs, which might notably down-regulate the activity of SKOR (Lacombe et al. 2000; Shi et al. 2002), accordingly, that extensive K^+ detained in XPCs might negatively regulate the expression of AKT1, reducing K^+ uptake by roots. The size of the *arrows* indicate the capabilities of proteins

XPCs is excreted to extracellular space by SOS1 and then diffuses into xylem to ameliorate cytoplasmic impairment by Na^+ (Shi et al. 2002). Na^+ would then flow quickly to the aerial part by transpiration pull, and over-accumulated Na^+ in leaves would certainly compromise the photosynthetic tissues (Pardo 2010). Sequestering a portion of Na^+ is a strategy to alleviate the damage. Besides, HKT localized in XPCs could retrieve part of Na^+ from xylem (Ren et al. 2005; Sunarpi et al. 2005; Byrt et al. 2007; Guo et al. 2012). The second pathway could depolarize membrane of XPCs and then induce K^+ loading to the xylem by depolarization-activated SKOR (Stele K^+ Outwardly Rectifying) and subsequently promotes K^+ transport to shoots (Gaymard et al. 1998; Horie et al. 2009; Guo et al. 2012). Plentiful K^+ transport to shoots, through feedback

regulation, stimulates the expression of *AKT1* located at epidermis, cortex and endodermis, promoting more K^+ acquisition from environments, which can, on the one hand, provide adequate K^+ nutrition for plants; and on the other hand, increase K^+/Na^+ ratio in cytoplasm and then mitigate the damage by toxic Na^+. Under the mild salt condition (Fig. 7b), Na^+ is loaded into xylem via SOS1 and then delivered to shoots since its accumulation do not exceed the capacity of the vacuoles compartmenting Na^+ (Blumwald et al. 2000; Shi et al. 2002; Guo et al. 2012). Meanwhile, hydrion is pumped into XPCs by SOS1, resulting in acidification of the cells, which might notably down-regulate the activity of SKOR (Lacombe et al. 2000), thereby negatively regulating the expression of AKT1 and decreasing K^+ uptake by roots. This might be the reason

why the 25 mM Na^+ showed higher repression than 150 mM Na^+.

In conclusion, the results in this paper suggest that PtAKT1 maintain a strong selective absorption capacity for K^+ over Na^+ during various salinity. We hypothesize a model to expound the different effects of various salinity on the expressions of *PtAKT1*, and provide a likely explanation to how SOS1 protect the AKT1 mediating K^+ influx during salt stress.

Author contribution statement Conceived and designed the experiments: Qiang Guo and Suo-Min Wang. Performed the experiments: Qiang Guo, Pei Wang and Xiang-Rui Zhou. Analyzed the data: Qiang Guo and Pei Wang. Performed the analysis with constructive discussions: Pei Wang and Suo-Min Wang. Wrote the paper: Pei Wang, Qian Wang and Suo-Min Wang.

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