DOI: 10.1007/s10535-014-0462-1 BIOLOGIA PLANTARUM 59 (1): 65-73, 2015

Transcription of potassium transporter genes of KT/HAK/KUP family in peach seedlings and responses to abiotic stresses

Z.-Z. SONG[1,2], Y. YANG[1], R.-J. MA[1], J.-L. XU[1], and M.-L. YU[1]*

Institute of Horticulture, Jiangsu Academy of Agricultural Sciences, and Jiangsu Key Laboratory of Horticultural Crop Genetic Improvement, Nanjing 210014, P.R. China[1]
State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, P.R. China[2]

Abstract

Potassium uptake and transport is facilitated by KT/HAK/KUP transporters. In this study, we identified 16 putative K^+-uptake transporter genes in peach (*Prunus persica*). To investigate the role of *PpeKUP* in maintaing K^+ uptake, transport, and homeostasis, we applied abiotic stresses to peach seedlings and analysed physiological reactions and transcriptional responses of *PpeKUP* genes. The peach seedlings were sensitive to polyethylene glycol (PEG), Pb, and Cd, as evidenced by impaired growth, K^+ nutrition, and photosynthetic performance. However, the peach seedlings were tolerant to aluminum. K^+ deficiency mainly increased, whereas K^+ excess reduced the *PpeKUP* gene expression in roots. The Al treatments enhanced the *PpeKUP* transcription in shoots, whereas PEG, Pd, and Cd enhanced the *PpeKUP* transcription in all tissues. Our findings provided molecular basis for K^+ uptake, transport, and homeostasis in the peach seedlings, and revealed potential candidate genes for further functional determination or breeding of peaches.

Additional key words: aluminum, cadmium, gene expression, lead, osmotic stress, polyethylene glycol, *Prunus persica*.

Introduction

As one of the most abundant cations in plant cells, potassium (K^+) contributes to many physiological and metabolic processes, such as neutralization of anions, maintenance of cellular osmolarity, and control of stomata opening (Véry and Sentenac 2003, Grabov 2007). K^+ deficiency negatively affects plant growth, photosynthesis, chlorophyll content, and chloroplast ultrastructure (Zhao *et al*. 2001, Ashley *et al*. 2006).

To meet growth demands, an optimal amount of K^+ must be absorbed effectively from soil *via* plant roots. In particular, K^+ transporters are famous for acquiring K^+, catalyzing K^+ uptake across a wide spectrum of external concentrations, mediating K^+ movement within the plant, as well as its efflux into the environment, and possibly maintaining K^+ homeostasis in plants (Véry and Sentenac 2003, Ashley *et al*. 2006, Grabov 2007). Individual members of the largest K^+ transporter family, K^+ transporter/high-affinity K^+ transporter/K^+ uptake permease (KT/HAK/KUP), have been found in many plants, *e.g*., *Arabidopsis* (Rubio *et al*. 2000, Mäser *et al*. 2001), rice (Bañuelos *et al*. 2002, Gupta *et al*. 2008), barley (Vallejo *et al*. 2005), tomato (Wang *et al*. 2002, Nieves-Cordones *et al*. 2007), alligator weed (Song and Su 2013), *etc.* Several members of the KT/HAK/KUP family have been characterised in plants, either by overexpression in *Arabidopsis* (Fu and Luan 1998, Song *et al*. 2014a) or by analysis of T-DNA insertion mutants (Rigas *et al*. 2001, Mian *et al*. 2011). The ubiquitous presence of these KT/HAK/KUP genes in plants implies that they play an important role in improving plant tolerance to drought (Li *et al*. 2011, Song and Su 2013, Song *et al.* 2014b), salinity (Mian *et al*. 2011, Upadhyay

Submitted 3 April 2014, *last revision* 21 May 2014, *accepted* 27 May 2014.
Abbreviations: ICP-AES - inductively coupled plasma - atomic emission spectrometry; Chl - chlorophyll; g_s - stomatal conductance; KT/HAK/KUP - K^+ transporter/high-affinity K^+ transporter/K^+ uptake permease; PEG - polyethylene glycol; P_N - net photosynthetic rate; RT-qPCR - real time quantitative polymerase chain reaction.
Acknowledgments: This work was supported by grants from the China Agriculture Resarch System (CAR-31), Jiangsu Agriculture Science and Technology Innovation Fund CX(15)2014, and the Jiangsu Postdoctoral Science Foundation (1401080B). The first two authors contributed equally to this work.
* Corresponding author, fax: (+86) 25 83490218, e-mail: mly1008@aliyun.com

et al. 2012, Bose *et al*. 2014, Song *et al.* 2014a), and cold (Rai *et al*. 2008, Ramalho *et al*. 2013).

Notably, K^+ is also closely related to flower formation, fruit quality, and fruit yield (Demiral and Köseoglu 2005, Hartz *et al*. 2005, Yurtseven *et al*. 2005, Davies *et al*. 2006, Nava *et al*. 2007, Leser *et al*. 2009). However, a molecular basis towards K^+ uptake and transport in fruit trees is largely rare. In particular, K^+ deficiency (Demiral and Köseoglu 2005, Hartz *et al*. 2005, Yurtseven *et al*. 2005), drought (Ozturk *et al*. 2002), and heavy metal stress (Worthington 2001, Lombardi and Sebastiani 2005) in orchards are increasingly the major challenges to fruit productivity and quality. How the K^+ uptake and transport genes respond to such abiotic stresses in fruit trees seems to be interesting and significant to set about.

As one of the most economically important fruit trees, peach (*Prunus persica*) has been genetically well-characterized (Jung *et al*. 2008, Layne and Bassi 2008) and Xiahui6 belongs to prominent Chinese cultivars (Yu *et al*. 2005). In this study, we identified 16 KUP family genes, analysed the expression of *KUP* genes and the physiological response of seedlings to K^+ deficiency, drought, and heavy metal stresses.

Materials and methods

Peach [*Prunus persica* (L.) Batsch cv. Xiahui6] stones obtained from the National Peach Germplasm Repository in Nanjing, China were washed and germinated in soil in a greenhouse. Seedlings with similar height were transferred to aerated plastic containers with a half strength Murashige and Skoog (1962; MS) solution containing 1 mM K^+. A temperature was 26 ± 2 °C, a relative humidity 60 %, a photoperiod 12 h, and an irradiance 1 000 μmol m^{-2} s^{-1}. The nutrient solution was changed every other day.

For K^+ deficiency treatments, K^+ was omitted from the MS medium. For K^+ excess treatments, germinated seedlings were grown in the 1/2 MS solution containing 20 mM K^+ (KCl, pH 5.8). In drought treatments, seedlings were grown in the 1/2 MS nutrient medium supplied with 10 % (m/v) polyethylene glycol (PEG 6 000, pH 5.8). In metal treatments, seedlings were grown in the 1/2 MS nutrient solution containing 200 μM Cd^{2+} ($CdCl_2$, pH 5.8), 500 μM Pb^{2+} [$Pb(NO_3)_2$, pH 5.8], or 200 μM Al^{3+} ($AlCl_3$, pH 4.5), respectively. The seedlings were exposed to the treatments for 72 h (for RT-qPCR determination) or 21 d (for physiological analysis), and then photographed.

The seedlings were collected and rinsed with distilled water and then weighed to obtain the fresh mass. Roots were scanned with an *Epson Rhizo* scanner (Long Beach, CA, USA), and the total root length and surface area were acquired with the *Epson WinRHIZO* software. For K^+ determination, samples were dried and digested using the HNO_3-$HCLO_4$ method and subjected to inductively coupled plasma - atomic emission spectrometry (ICP-AES; *IRIS Advantage*, *Thermo Electron*, Waltham, MA, USA).

Stomatal conductance (g_s) and net photosynthetic rate (P_N) at the terminal leaflet of the fully expanded second leaf were measured with a portable photosynthetic system *LI-6400* (*Li-COR,* Lincoln, NE, USA) according to Kumar *et al*. (2006). An irradiance was 1 000 μmol m^{-2} s^{-1}, a CO_2 concentration 400 μmol mol^{-1}, a temperature 25 °C, and a relative humidity 60 %.

Chlorophyll (Chl) was extracted as described by Song *et al*. (2014a). Fresh peach leaves were kept in 95 % (v/v) ethanol at 4 °C in darkness for 12 h, and then centrifuged at 1 000 *g* and 4 °C for 10 min. The supernatant was used to determine the absorbances of Chl *a* at 665 and Chl *b* at 649 nm in a *BioRad SmartSpec 3000* spectrophotometer (Wadsworth, IL, USA).

Free proline content was determined spectrophotometrically according to a previously described method (Song *et al*. 2014b). Fresh leaf tissue (0.5 g) was homogenised in 5 cm^3 of 3 % (m/v) sulphosalicylic acid at 95 °C for 15 min. After centrifugation, 2 cm^3 of the supernatant was transferred into a new tube containing 2 cm^3 of acetic acid and 2 cm^3 of an acidified ninhydrin reagent and incubated at 95 °C for 30 min. Then, 5 cm^3 of toluene was added to the tube. The absorbance of red products in the toluene layer was determined at 532 nm in the *BioRad SmartSpec 3000* spectrophotometer.

Total RNA was extracted from leaves, stems, or roots using a plant RNA kit (*BioTeKe*, Beijing, China). Extracted RNA was treated with a genomic DNA eraser to remove gDNA contamination and was reverse transcribed into cDNA using a *PrimeScript*^{TM} *RT* reagent kit (*TaKaRa*, Kyoto, Japan). Specific primers for *KUP* transporter genes and a control gene *ubiquitin* of peach were designed using a *NCBI/Primer-BLAST* on-line server (Table 1). Real-time quantitative PCR (RT-qPCR) was carried out in a *7500 Real Time PCR* system (*Applied Biosystems*, New York, USA). PCR conditions for thermal cycling were as follows: 95 °C for 30 s, 40 cycles of 95 °C for 5 s, and 60 °C for 34 s. The relative expressions of the target genes were presented after normalization to the internal control from three independent biological repeats.

The amino acid sequences of the *Arabidopsis* KT/HAK/KUP family were obtained from the *TAIR* database (http://www.arabidopsis.org/browse/genefamily/index.jsp). These sequences were used as query to the *BLAST* peach genome to identify peach orthologues. Coding sequences (CDS) of the identified putative KT/HAK/KUP family genes in peach were obtained in the genome database for *Rosaceae*. Protein domains were

verified by using the *InterProScan 4.8* web server (http://www.ebi.ac.uk/Tools/pfa/iprscan/). A multiple alignment analysis between peach and *Arabidopsis* KUP proteins was carried out using the *Clustal W* program built in the *MEGA 4.0* software. A phylogenetic tree was constructed by using the *MEGA 4.0* software between candidate KUP proteins in peach and *Arabidopsis*. The peach KUP orthologues were named by taking corresponding *Arabidopsis* KUP proteins as reference, based on the phylogenetic tree results.

For all experiments, data were statistically analysed using the independent samples *t*-test in the *SPSS 13.0* software (*SPSS*, Chicago, IL, USA). Data were compared between plants under the control and stress treatments. Details are described in the figure legends. The graphs were produced using the *Origin 8.0* software.

Table 1. Specific primers used for quantitative RT-PCR.

Gene	Acc. No.	Primers (5' to 3') forward	Primers (5' to 3') reverse	Amplicon size [bp]
PpeKUP1	KJ585786	AGGCGTTGAAGGATGGGTTT	GGCCAAAACACAGCTTCTGG	234
PpeKUP2	KJ585787	TAGAGGAGACTCAACCGGCA	CGAGTATAAACGCCGTCCCA	213
PpeKUP3	KJ585788	GTGCATCGTCAGGTATGGGT	GATGGAGTCGCTCACACCAA	235
PpeKUP4	KJ585789	AACTCCTGAAGGTGCTGTGG	TAGGTGGCCTGCAAGTTCTG	182
PpeKUP5	KJ585790	AGCGGAGGAAGGAGGTTAGT	GTGATACCCCACTTGCCACA	165
PpeKUP6	KJ585791	CCGACCCAACGAAGGAAGAA	TACGCTGAGTGCATAGCTGG	240
PpeKUP7	KJ585792	GACAATCAGAGCACCGGGAA	GACACGCCGGAAAAGAAACC	181
PpeKUP8	KJ585793	TGGGTCCCCATTGCTCTTTC	CGGACCCTAACAATCCCCAG	152
PpeKUP9	KJ585794	GTCAATCAAGGTGGGTGGGT	GGCGGGAAGGTTGGTGATAA	246
PpeKUP10	KJ585795	AATTGGCAATGCCTCTGGGA	GCGATCACAAGAGGAACCCA	204
PpeKUP11	KJ585796	TGCCTGCGATCCATTCTGTT	CATCCGAGTCTGAACACCCC	249
PpeKUP12	KJ585797	TTGTCCAAGGCATCCCATCC	CGGGCTATACAGCGGAACAT	178
sPpeKUP13	KJ585798	AGTCTGTGAGCTTGGTGAGC	AAGTACGCTGTGCCACTCTC	196
PpeKUP14	KJ585799	CGGAAAGATTGGCAACGCAT	AGAGGAAGGAAACCGCCTTG	201
PpeKUP15	KJ585800	CATACCTTCCGTCCACTCCG	TCAACCCTGTCGTTGTAGCC	159
PpeKUP16	KJ585801	GGCATACACTGGACAAGCCT	CCTGGCCGATGTGTGCACGA	226
Ubiquitin	KJ598788	AGGCTAAGATCCAAGACAAAGAG	CCACGAAGACGAAGCACTAAG	145

Results

Taking 13 *Arabidopsis* KT/HAK/KUP member sequences as references, 16 putative KT/HAK/KUP genes in peach (entitled as *PpeKUP*) were identified in the peach genome of the genome database for *Rosaceae* (Fig. 1). Protein domain verification shows that all peach KUP proteins possessed a K^+ transmembrane transporter activity. Names and Genebank acc. Nos. of the 16 *PpeKUP* genes are shown in Table 1. Notably, the peach genome encodes more KT/HAK/KUP family members than the *Arabidopsis* genome suggesting that trees probably depend more on K^+ uptake and transport. The phylogenetic analysis indicates that the KT/HAK/KUP family in peach can be classified into three groups (Fig. 1). In particular, peach PpeKUP1, PpeKUP2, PpeKUP3, PpeKUP4, PpeKUP6, PpeKUP7, PpeKUP8, PpeKUP11, and PpeKUP12 were closely clustered with corresponding *Arabidopsis* orthologues (Fig. 1) indicating that the KT/HAK/KUP family genes are highly conserved across higher plants.

To verify the expression profiles of putative *PpeKUP* genes in peach, we performed RT-qPCR determination. The results show that 16 *PpeKUP* genes were unevenly expressed in leaves, stems, and roots. In particular, *PpeKUP6* was highly expressed in leaves, more than in roots and stems, whereas *PpeKUP4* and *PpeKUP16* were expressed more in stems, and the expressions of *PpeKUP1*, *PpeKUP5*, *PpeKUP7*, *PpeKUP7*, and *PpeKUP8* were higher in roots. The remaining eight genes were evenly expressed throughout entire peach plants (Fig. 2). Notably, the *PpeKUP5* and *PpeKUP8* genes had the highest expression in roots indicating that these genes encode dominant K^+ transporters in peach roots.

To investigate *PpeKUP* gene expression profiles in peach seedlings under stresses, we applied a PEG-induced drought stress, and an excess of Pb, Cd, or Al. All these stresses significantly affected the peach seedlings (Fig. 1 Suppl.). RT-qPCR indicates that the *PpeKU*P genes were differentially regulated by these abiotic stresses (Table 1 Suppl.).

K^+ is an indispensible in plant nutrition, and K^+ deficiency inhibits plant growth (Ashley *et al*. 2006). However, the K^+ deficiency caused mild changes in the phenotype of the peach seedling (Fig. 1*A* Suppl.).

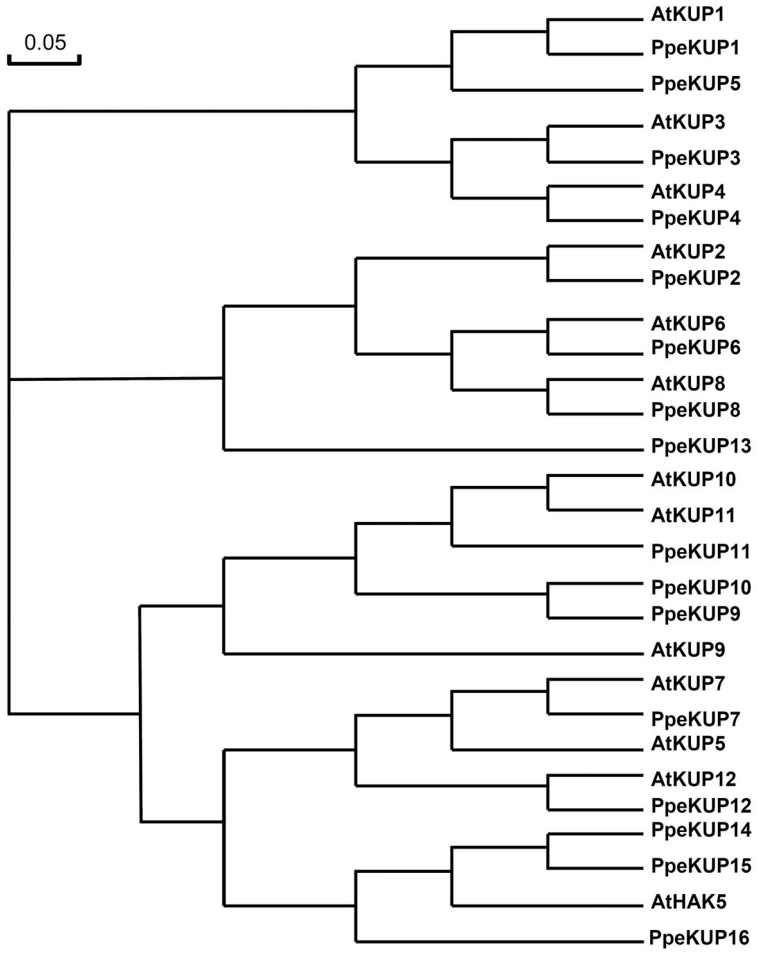

Fig. 1. The phylogenetic tree of KT/HAK/KUP family proteins from peach and *Arabidopsis*. The tree was created by the bootstrap option of the *CLUSTAL W* multiple alignment packages and the neighbour-joining method using 16 members of peach and 13 members of *Arabidopsis* KT/HAK/KUP amino acid sequences. The *scale* indicates the genetic distance. Acc. Nos. of 13 KUP/KT/HAK family members in *Arabidopsis* are: AtKUP1, NP_180568.1, AtKUP2, NP_565936.1, AtKUP3, NP_186854.1, AtKUP4, NP_194095.2, AtKUP5, NP_195079.2, AtKUP6, NP_177187.2, AtKUP7, NP_568213.2, AtKUP8, NP_196992.1, AtKUP9, NP_193729.1, AtKUP10, NP_174397.1, AtKUP11, NP_181051.1, AtKUP12, NP_176222.2, and AtHAK5, and NP_567404.1.

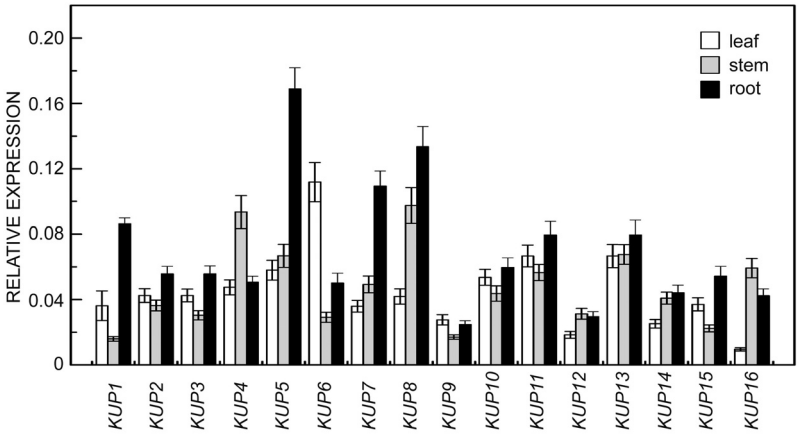

Fig. 2. The expression profiles of *PpeKUP* genes. Seedlings were grown in a 1/2 MS solution (1 mM K^+, control conditions) for 3 d before RT-qPCR examination. Expression values are given as ratio relative to the values of *actin*. Data are means of values obtained from three independent replicates.

Compared to the controls, the shoot fresh mass was not significantly changed under the K^+ deficiency, whereas the root fresh mass was reduced by approximately 28 %, and the seedlings had more chlorotic leaves (Fig. 3*A*). However, there were no significant differences in either the total root length or the root surface area (Fig. 4*A*). In particular, K^+ accumulated more in shoots than in roots, and the K^+ deficiency definitely decreased the tissue K^+ concentration throughout the whole plant (Fig. 5*A*). Correspondingly, P_N and g_s were significantly reduced

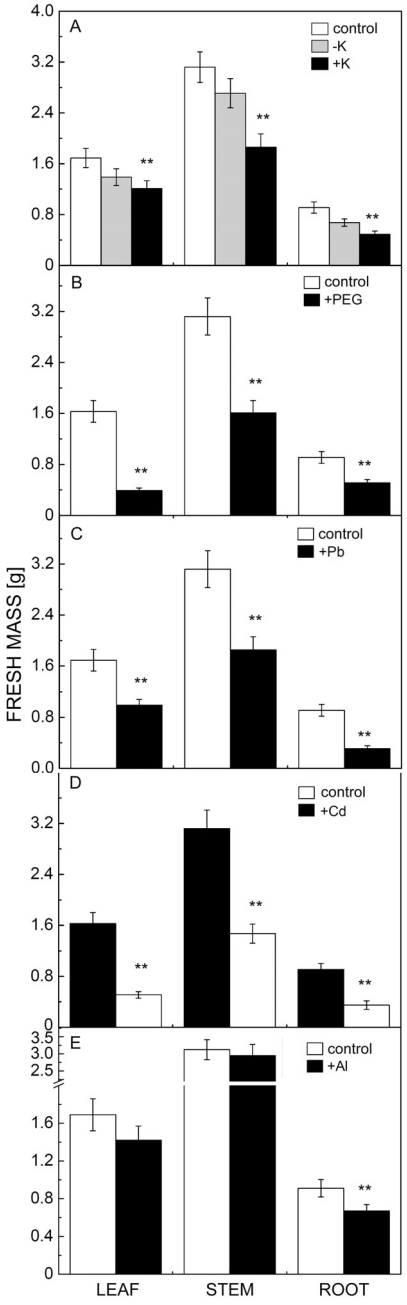

Fig. 3. The analysis of plant fresh mass. *A* - K^+ treatment; *B* - PEG treatment; *C* - Pb treatment; *D* - Cd treatment; *E* - Al treatment. Seedlings were exposed to the treatments for 21 d before examination. –K, +K, +PEG, +Pd, +Cd, and +Al indicate K^+ deficiency, K^+ excess, 10 % PEG (pH 5.8), 500 μM $Pb(NO_3)_2$ (pH 5.8), 200 μM $CdCl_2$ (pH 5.8), and 200 μM $AlCl_3$ (pH 4.5), respectively. Means ± SE, n =18. The *asterisks* indicate significant differences between the control and the stress treatment ($P < 0.01$, the independent-samples t-test).

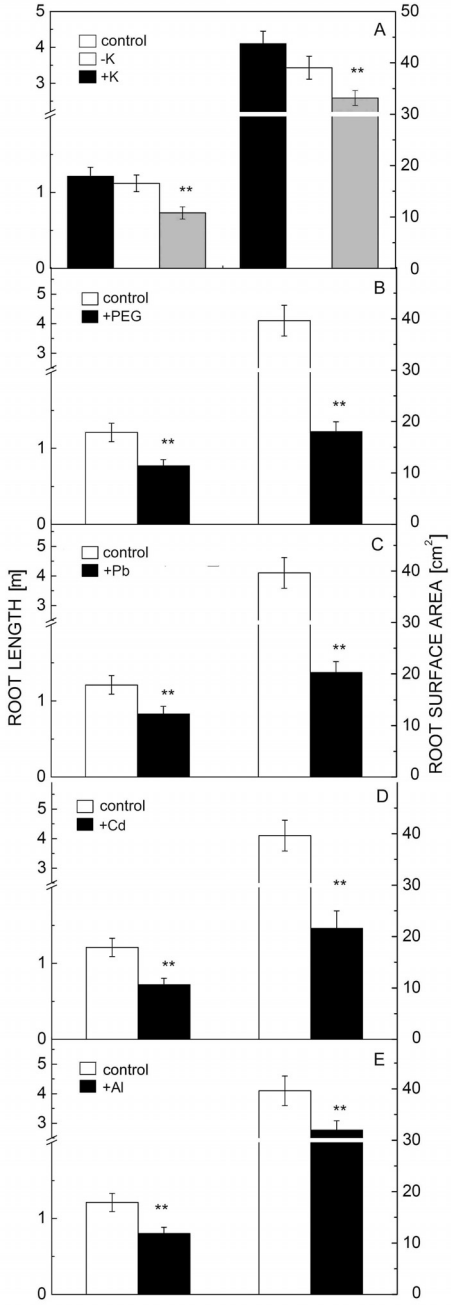

Fig. 4. Root length and surface area assays. *A* - K^+ treatment; *B* - PEG treatment; *C* - Pb treatment; *D* - Cd treatment; *E* - Al treatment. Seedlings were exposed to the treatments for 21 d before examination. –K, +K, +PEG, +Pd, +Cd, and +Al indicate K^+ deficiency, K^+ excess, 10 % PEG (pH 5.8), 500 μM $Pb(NO_3)_2$ (pH 5.8), 200 μM $CdCl_2$ (pH 5.8), and 200 μM $AlCl_3$ (pH 4.5), respectively. Means ± SE, n =18. The *asterisks* indicate significant differences between the control and the stress treatment ($P < 0.01$, the independent-samples t-test).

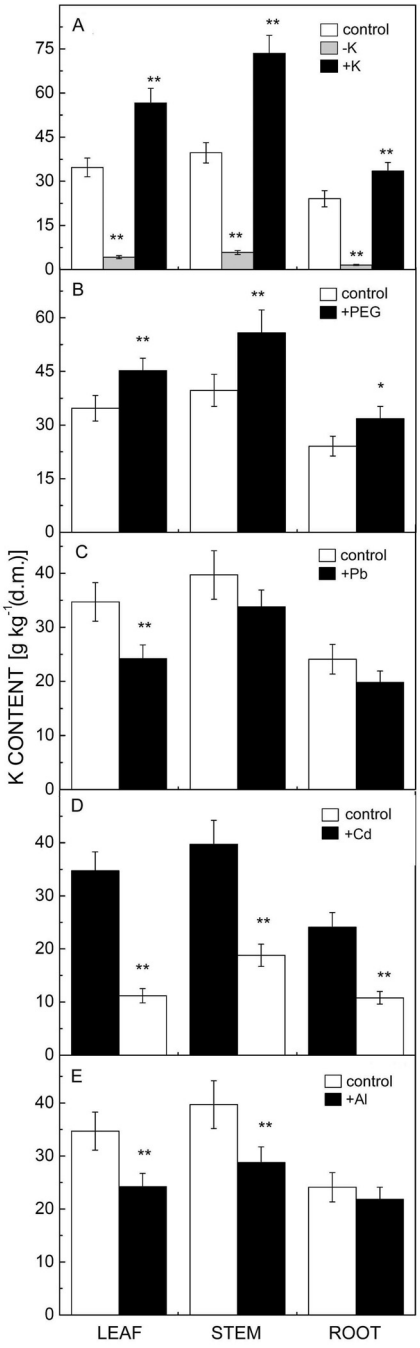

Fig. 5. Tissue K^+ content determination. *A* - K^+ treatment; *B* - PEG treatment; *C* - Pb treatment; *D* - Cd treatment; *E* - Al treatment. Seedlings were exposed to the treatments for 21 d before examination. –K, +K, +PEG, +Pd, +Cd, and +Al indicate K^+ deficiency, K^+ excess, 10 % PEG (pH 5.8), 500 μM $Pb(NO_3)_2$ (pH 5.8), 200 μM $CdCl_2$ (pH 5.8), and 200 μM $AlCl_3$ (pH 4.5), respectively. Means ± SE, n =18. The *asterisks* indicate significant differences between the control and the stress treatment (* - $P < 0.05$, ** - $P < 0.01$, the independent-samples *t*-test).

(Table 2). Simultaneously, the total leaf Chl content was reduced (Table 2) which explained the chlorotic leaves. The K^+ deficiency caused little change in the leaf proline content (Table 2). In addition, the expressions of 10 out of 16 *PpeKUP* genes were affected by the K^+ deficiency (Fig. 6*A*). In particular, the K^+ deficiency enhanced the expression of eight genes in roots, whereas The expression of *PpeKUP6* was dramatically reduced in all the tested tissues (Fig. 6*A*). The K^+ excess remarkably inhibited shoot and root growth (Fig. 1*A* Suppl.). The fresh masses of leaves, stems, and roots were reduced approximately by 28, 40, and 56 %, respectively (Fig. 3*A*). The total root length, root surface area, P_N, g_s, and Chl content were also significantly reduced (Fig. 4*A*, Table 2), but there was no significant difference in the leaf proline accumulation (Table 2). The K^+ excess largely decreased the expression of nine *PpeKUP* genes in roots, but significantly enhanced the *PpeKUP1* and *PpeKUP2* expressions (Fig. 6*B*). Notably, *PpeKUP6* and *PpeKUP8* were most sensitive to excess K^+, their expressions were obviously reduced in all the tested organs (Fig. 6*B*). Even so, the tissue K^+ accumulation significantly increased, especially in shoots (Fig. 5*A*).

The PEG treatment caused the highest stress (Fig. 1*B* Suppl.). The total fresh mass decreased by nearly 64 % (Fig. 3*B*), and also the root length, root surface area, P_N, g_s, and Chl content were greatly reduced (Fig. 4*B*, Table 2). In particular, the PEG treatment significantly enhanced the K^+ content especially in aboveground parts (Fig. 5*B*) and also the proline content (Table 2). The expressions of *PpeKUP* genes mostly increased, except for *PpeKUP3* and *PpeKUP12* that decreased (Fig. 6*C*). Six genes (*PpeKUP1*, *PpeKUP4*, *PpeKUP5*, *PpeKUP6*, *PpeKUP13*, and *PpeKUP16*) had the highest expression in all the tested organs (Fig. 6*C*). In particular, *PpeKUP5* was up-regulated 3-, 7- and 6-fold in leaves, stems, and roots, respectively (Fig. 6*C*).

The toxic heavy metals, Pb and Cd, caused severe stress (Fig. 1*C* Suppl.). Under 500 μM Pb^{2+}, the fresh masses of leaves, stems, and roots were reduced by approximately 39, 42, and 66 %, respectively (Fig. 3*C*). They were also reduced under 200 μM Cd^{2+} by approximately 60, 51, and 59 %, respectively, (Fig. 3*D*). Also the total root length, root surface area, P_N, g_s, Chl content, and K^+ accumulation were greatly reduced, whereas the proline content was enhanced at the Pb^{2+} or Cd^{2+} stress (Fig. 4*C*, Table 2). The excess of Pb largely enhanced the expression of nine *PpeKUP* genes throughout the whole plant but significantly reduced the *PpeKUP6* and *PpeKUP10* expressions (Fig. 6*D*). In particular, *PpeKUP2* was greatly up-regulated 6-, 10-, and 7-fold in leaves, stems, and roots, respectively. In contrast, *PpeKUP6* was down-regulated almost 6-fold in roots and 3-fold in shoots (Fig. 6*D*). Totally, 13 genes were responsive to the Cd treatment; their expressions were mainly induced with the exception of *PpeKUP5* and *PpeKUP7* whose expressions decreased (Fig. 6*E*). Particularly, the expressions of *PpeKUP3*, *PpeKUP6*, and *PpeKUP10* significantly enhanced in all the tested

tissues, whereas the *PpeKUP7* expression was obviously reduced throughout the whole plant (Fig. 6*E*).

Interestingly, 200 μM Al^{3+} was not very toxic to the peach seedlings (Fig. 1*C* Suppl.). The fresh masses of leaves and stems were not changed, whereas the fresh mass, length, and surface area of roots decreased (Fig. 3*E*, 4*E*). It is worth mentioning that P_N, g_s, and Chl and proline content were not significantly changed compared to the control conditions (Table 2). Moreover, the shoot K^+ content was obviously reduced under the Al treatment (Fig. 5*E*). Notably, nine genes were responsive to the Al treatment and their expressions increased (especially in above-ground parts) except those for *PpeKUP6* in shoots and *PpeKUP13* in leaves that decreased (Fig. 6*F*). Particularly, the expression of *PpeKUP3* significantly enhanced in all the tested tissues, whereas the expression of *PpeKUP6* was reduced in shoots but increased in roots (Fig. 6*F*).

Table 2. The effects of different treatments [K^+ deficiency, K^+ excess, 10 % PEG, 500 μM $Pb(NO_3)_2$, 200 μM $CdCl_2$, and 200 μM $AlCl_3$] lasting 21 d on net photosynthetic rate, stomatal conductance, chlorophyll content, and proline content in peach seedlings. Means ± SE, n = 36. The *asterisks* indicate significant differences between the control and the stress treatment (* - $P < 0.05$, ** - $P < 0.01$, the independent samples t-test).

Treatments	P_N [μmol m^{-2} s^{-1}]	g_s [mmol m^{-2} s^{-1}]	Chl [g kg^{-1}(f.m.)]	Proline [g kg^{-1}(f.m.)]
Control	9.45 ± 0.31	0.24 ± 0.02	1.32 ± 0.12	13.72 ± 1.22
-K	7.82 ± 0.25*	0.19 ± 0.02**	0.91 ± 0.10*	11.61 ± 1.24
+K	7.67 ± 0.25*	0.17 ± 0.01**	0.94 ± 0.11*	15.83 ± 1.71
+PEG	2.35 ± 0.13**	0.07 ± 0.01**	0.33 ± 0.04**	23.83 ± 3.04**
+Pb	4.54 ± 0.17**	0.09 ± 0.01**	0.70 ± 0.06**	19.66 ± 1.86**
+Cd	3.93 ± 0.12**	0.11 ± 0.02**	0.51 ± 0.04**	24.31 ± 2.67**
+Al	8.54 ± 0.36	0.21 ± 0.02	1.19 ± 0.14	15.66 ± 1.46

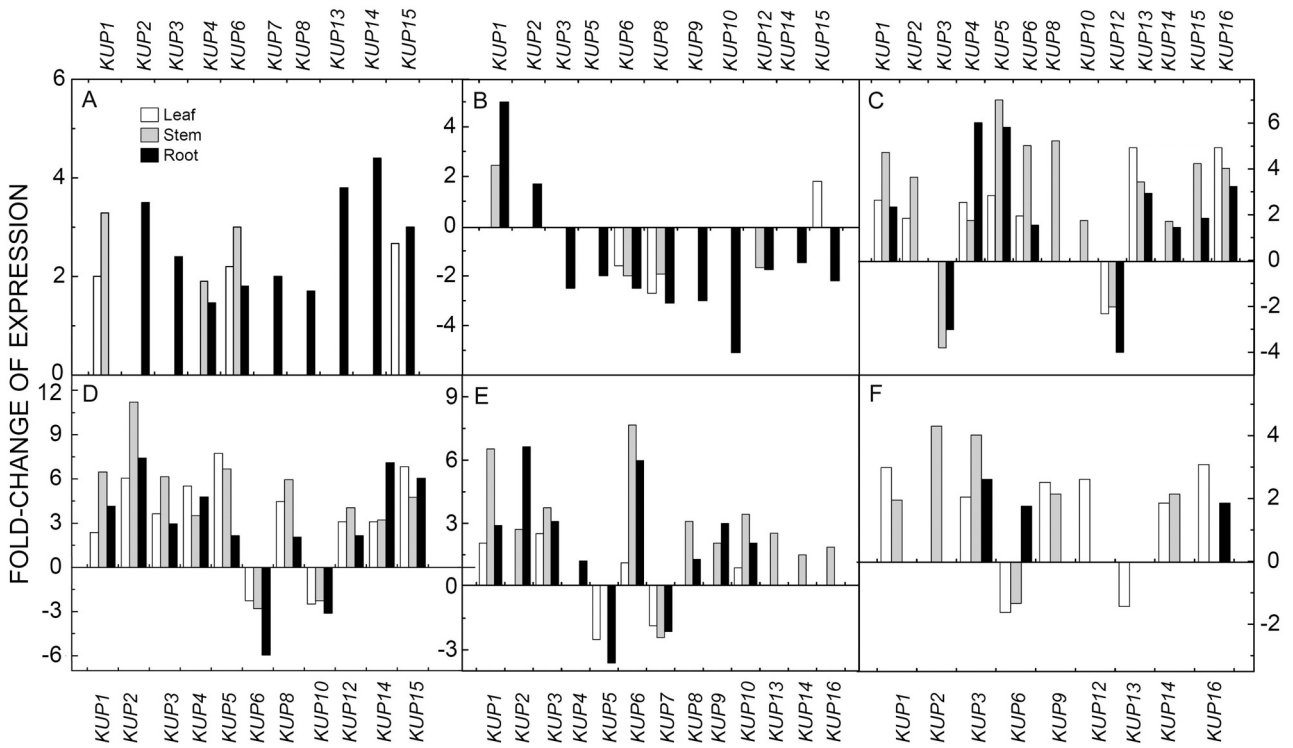

Fig. 6. Expression changes of *PpeKUP* genes under different treatments. *A* - K^+ deficiency; *B* - K^+ excess; *C* - PEG treatment; *D* - Pb treatment; *E* - Cd treatment; *F* - Al treatment;. Seedlings were exposed to the treatments for 72 h before qRT-PCR determination. –K, +K, +PEG, +Pd, +Cd, and +Al indicate K^+ deficiency, K^+ excess, 10 % PEG (pH 5.8), 500 μM $Pb(NO_3)_2$ (pH 5.8), 200 μM $CdCl_2$ (pH 5.8), and 200 μM $AlCl_3$ (pH 4.5), respectively. The relative expressions of genes were presented after normalization to the internal control from three independent biological repeats.

Discussion

Among plant mineral nutrients, K^+ stands out as cation having the strongest influence on fruit quality attributes (Leser *et al*. 2009). In agriculture, the application of K^+ fertilizers efficiently promotes fruit yield and improves fruit quality. However, many plant, soil, and environmental factors often limit adequate K^+ uptake from soil to satisfy the requirements during plant development. To strengthen K^+ uptake and transport is urgent for plants under adverse environments.

In the present study, we identified 16 K^+-uptake transporters in peach. The RT-qPCR results show that the *PpeKUP* genes were differentially regulated by the abiotic stresses, *e.g.*, the K^+ depletion mainly enhanced, whereas the K^+ excess reduced the expressions of *PpeKUP* genes in roots; the excess of Al enhanced the expressions of *PpeKUP* genes in aboveground parts, whereas the PEG, Pb, and Cd stresses mostly enhanced the *PpeKUP*s transcriptions with no tissue specificity (Fig. 6). Interestingly, most of the *PpeKUP* genes responded to at least one treatment (Table 1 Suppl.). In particular, *PpeKUP1*, *PpeKUP2*, *PpeKUP3*, *PpeKUP6*, and *PpeKUP14* genes, whose expressions were affected by all the treatments (Fig. 6, Table 1 Suppl.), were more likely involved in maintaining K^+ homeostasis under the adverse conditions. Notably, *PpeKUP6* was the most sensitive gene throughout the whole plant which responded to all the treatments . In contrast, *PpeKUP11* remained unaffected after all the treatments. Possibly, this gene persistently contributed to the peach root absorption and transport of K^+, at least during seedling stages. Moreover, the highly-expressed gene was *PpeKUP5* in roots (Fig. 2), implying that this gene is probably driven by a stronger promoter.

K^+ deficiency negatively affects plant growth and activates the expression of high affinity transporters (Ashley *et al*. 2006). In this study, a majority of the *PpeKUP* genes were activated by the K^+ deficiency, which might be closely involved in maintaining the maximal uptake of external K^+ and might be a major mechanism of adaptation to K^+ deficiency. However, the excessive amount of K^+ limited normal plant growth (Fig. 1*A* Suppl.), affected the internal K^+ content and so K^+-dependent metabolic pathways or cellular processes. The expressions of a half of the *PpeKUP* genes largely decreased in plant roots under the K^+ excess suggesting that the peach seedlings utilised K^+ less efficiently and reduced transport systems to avoid K^+ toxicity.

The PEG treatment or excess of heavy metals inhibited root development resulting in a decreased root surface area. Under such stresses, seedlings mobilized K^+ transporters (the expression of *PpeKUP* genes) to maximize the root uptake and accumulation of K^+ necessary for vital activities including phytosynthesis and stomatal movement. In addition, in plants under stress conditions, stress tolerance mechanisms including the proline accumulation arise (Table 2), which contributes to the osmotic adjustment and enzyme stabilization (Choudhary *et al*. 2007, Nounjan *et al*. 2012). Although annual plants like onion (Achary *et al*. 2012) and wheat (Basu *et al*. 1994) are sensitive to 100 μM Al^{3+}, the peach seedlings tolerated 200 μM Al^{3+}.

In conclusion, we identified 16 KT/HAK/KUP family genes which differentially contributed to K^+ homeostasis and stress tolerance of the peach seedlings. Our findings not only provided a molecular basis for K^+ uptake and transport systems in peach, but also revealed potential candidate genes for further functional determinations. Currently, the question of how the *PpeKUP* genes behave during peach fruit development is under way.

References

Achary, V.M., Parinandi, N.L., Panda, B.B.: Aluminum induces oxidative burst, cell wall NADH peroxidase activity, and DNA damage in root cells of *Allium cepa* L. - Environ. mol. Mutagen. **53**: 550-60, 2012.

Ashley, M.K., Grant, M., Grabov, A.: Plant responses to potassium deficiencies: a role for potassium transport proteins. - J. exp. Bot. **57**: 425-436, 2006.

Bañuelos, M.A., Garciadeblas, B., Cubero, B., Rodríguez-Navarro, A.: Inventory and functional characterization of the HAK potassium transporters of rice. - Plant Physiol. **130**: 784-794, 2002.

Basu, U., Godbold, D., Taylor, G.J.: Aluminum resistance in *Triticum aestivum* associated with enhanced exudation of malate. - J. Plant Physiol. **144**: 747-753, 1994.

Bose, J., Rodrigo-Moreno, A., Shabala, S.: ROS homeostasis in halophytes in the context of salinity stress tolerance. - J. exp. Bot. **65**: DOI:10.1093/jxb/ert430, 2014.

Choudhary, M., Jetley, U.K., Abash Khan, M., Zutshi, S., Fatma, T.: Effect of heavy metal stress on proline, malondialdehyde, and superoxide dismutase activity in the cyanobacterium *Spirulina platensis*-S5. - Ecotox. environ. Safety **66**: 204-209, 2007.

Davies, C., Shin, R., Liu, W., Thomas, M.R.: Transporters expressed during grape berry (*Vitis vinifera* L.) development are associated with an increase in berry size and berry potassium accumulation. - J. exp. Bot. **57**: 3209-3216, 2006.

Demiral, M.A., Köseoglu, A.T.: Effect of potassium on yield, fruit quality, and chemical composition of greenhouse-grown aalia melon. - J. Plant Nutr. **28**: 93-100, 2005.

Fu, H.H., Luan, S.: AtKuP1: a dual-affinity K^+ transporter from *Arabidopsis*. - Plant Cell **10**: 63-73, 1998.

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

Gupta, M., Qiu, X., Wang, L., Xie, W., Zhang, C.J., Xiong, L.Z., Lian, X.M., Zhang, Q.F.: KT/HAK/KUP potassium transporters gene family and their whole-life cycle expression profile in rice (*Oryza sativa*). - Mol. Genet. Genomics **280**: 437-452, 2008.

Hartz, T.K., Johnstone, P.R., Francis, D.M., Miyao, E.M.: Processing tomato yield and fruit quality improved with potassium fertigation. - HortScience **40**: 1862-1867, 2005.

Jung, S., Staton, M., Lee, T., Blenda, A., Svancara, R., Abbott, A., Main, D.: GDR (Genome Database for Rosaceae): integrated web-database for *Rosaceae* genomics and genetics data. - Nucl. Acids Res. **36**: D1034-D1040, 2008.

Kumar, N., Kumar, S., Vats, S.K., Ahuja, P.S.: Effect of altitude on the primary products of photosynthesis and the associated enzymes in barley and wheat. - Photosynth. Res. **88**: 63-71, 2006.

Layne, D.R., Bassi, D.: The Peach: Botany, Production and Uses. - CABI, London 2008.

Leser, G.E., Jifon, J.L., Makus, D.J.: Impact of potassium nutrition on postharvest fruit quality: melon (*Cucumis melo* L) case study. - Plant Soil **335**: 117-131, 2009.

Li, M., Li, Y., Li, H., Wu, G.: Overexpression of *AtNHX5* improves tolerance to both salt and drought stress in *Broussonetia papyrifera* (L.) Vent. - Tree Physiol. **31**: 349-357, 2011.

Lombardi, L., Sebastiani, L.: Copper toxicity in *Prunus cerasifera*: growth and antioxidant enzymes responses of *in vitro* grown plants. - Plant Sci. **168**: 797-802, 2005.

Mäser, P., Thomine, S., Schroeder, J.I., Ward, J.M., Hirschi, K., Sze, H., Talke, I.N., Amtmann, A., Maathuis, F.J., Sanders, D., Harper, J.F., Tchieu, J., Gribskov, M., Persans, M.W., Salt, D.E., Kim, S.A., Guerinot, M.L.: Phylogenetic relationships within cation transporter families of *Arabidopsis*. - Plant Physiol. **126**: 1646-1667, 2011.

Mian, A., Oomen, R.J., Isayenkov, S., Sentenac, H., Maathuis, F.J., Véry, A.A.: Over-expression of an Na^+-and K^+-permeable HKT transporter in barley improves salt tolerance. - Plant J. **68**: 468-479, 2011.

Murashige, T., Skoog, F.: A revised medium for rapid growth and bioassays with tobacco tissue cultures. - Physiol. Plant **15**: 473-497, 1962.

Nava, G., Dechen, A.R., Nachtigall, R.G.: Nitrogen and potassium fertilization affect apple fruit quality in Southern Brazil. - Commun. Soil Sci. Plant. **39**: 96-107, 2007.

Nieves-Cordones, M., Martinez-Cordero, M.A., Martinez, V., Rubio, F.: An NH_4^+-sensitive component dominates high-affinity K^+ uptake in tomato plants. - Plant Sci. **172**: 273-280, 2007.

Nounjan, N., Nghia, P.T., Theerakulpisut, P.: Exogenous proline and trehalose promote recovery of rice seedlings from salt-stress and differentially modulate antioxidant enzymes and expression of related genes. - J. Plant Physiol. **169**: 596-604, 2012.

Ozturk, Z.N., Talame, V., Deyholos, M., Michalowski, C.B., Galbraith, D.W., Gozukirmizi, N., Tuberosa, R., Bohnert, H.J.: Monitoring large-scale changes in transcript abundance in drought- and salt-stressed barley. - Plant mol. Biol. **48**: 551-573, 2002.

Rai, R.K., Singh, P., Shrivastava, A.K., Suman, A.: Modulation of low-temperature-induced biochemical changes in bud and root band zones of sugar cane sets by potassium, zinc, and ethrel for improving sprouting. - J. Agr. Food Chem. **56**: 11976-11982, 2008.

Ramalho, J.C., Fortunato, A.S., Goulao, L.F., Lidon, F.C.: Cold-induced changes in mineral content in leaves of *Coffea* spp. Identification of descriptors for tolerance assessment. - Biol. Plant. **57**: 495-506, 2013.

Rigas, S., Debrosses, G., Haralampidis, K., Vicente-Agullo, F., Feldmann, K.A., Grabov, A., Dolan, L., Hatzopoulos, P.: *Trh1* encodes a potassium transporter required for tip growth in *Arabidopsis* root hairs. - Plant Cell **13**: 139-151, 2001.

Rubio, F., Santa-Maria, G.E., Rodriguez-Navarro, A.: Cloning of *Arabidopsis* and barley cDNAs encoding HAK potassium transporters in root and shoot cells. - Physiol. Plant **109**: 34-43, 2000.

Song, Z.Z., Su, Y.H.: Distinctive potassium-accumulation capability of alligator weed (*Alternanthera philoxeroides*) links to high-affinity potassium transport facilitated by K^+-uptake systems. - Weed Sci. **61**: 77-84, 2013.

Song, Z.Z., Yang, S.Y., Zhu, H., Jin, M., Su, Y.H.: Heterologous expression of an alligator weed high-affinity potassium transporter gene enhances salinity tolerance in *Arabidopsis*. - Amer. J. Bot. **101**: 840-850, 2014a.

Song, Z.Z., Yang, S.Y., Zuo, J., Su, Y.H.: Over-expression of *ApKUP3* enhances potassium nutritional status and drought tolerance in transgenic rice. - Biol. Plant. **58**: 649-658, 2014b.

Upadhyay, A., Upadhyay, A.K., Bhirangi, R.A.: Expression of Na^+/H^+ antiporter gene in response to water and salinity stress in grapevine rootstocks. - Biol. Plant. **56**: 762-766, 2012.

Vallejo, A.J., Peralta, M.L., Santa-Maria, G.E.: Expression of potassium-transporter coding genes, and kinetics of rubidium uptake, along a longitudinal root axis. - Plant Cell Environ. **28**: 850-862, 2005.

Véry, A.A., Sentenac, H.: Molecular mechanisms and regulation of K^+ transport in higher plants. - Annu. Rev. Plant Biol. **54**: 575-603, 2003.

Wang, Y.H., Garvin, D.F., Kochian, L.V.: Rapid induction of regulatory and transporter genes in response to phosphorus, potassium, and iron deficiencies in tomato roots. Evidence for cross talk and root/rhizosphere-mediated signals. - Plant Physiol. **130**: 1361-1370, 2002.

Worthington, V.: Nutritional quality of organic versus conventional fruits, vegetables, and grains. - J. Altern. Complem. Med. **7**: 161-173, 2001.

Yu, M.L., Ma, R.J., Du, P., Song, H.F., Shen, Z.J., Zhang, Y., Xu, J.L.: [Breeding report on mid-season peach cultivar Xiahui 6.] - J. Fruit Sci. **22**: 298-299, 2005. [In Chinese]

Yurtseven, E., Kesmez, G.D., Ünlükara, A.: The effects of water salinity and potassium levels on yield, fruit quality and water consumption of a native Central Anatolian tomato species (*Lycopersicon esculantum*). - Agr. Water Manage. **78**: 128-135, 2005.

Zhao, D., Oosterhuis, D.M., Bednarz, C.W.: Influence of potassium deficiency on photosynthesis, chlorophyll content, and chloroplast ultrastructure of cotton plants. - Photosynthetica **39**: 103-109, 2001.