



Genome-wide analysis and identification of KT/HAK/KUP potassium transporter gene family in peach (*Prunus persica*)

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ABSTRACT. The KT/HAK/KUP family members encoding high-affinity potassium (K^+) transporters mediate K^+ transport across the plasma membranes of plant cells to maintain plant normal growth and metabolic activities. In this paper, we identified 16 potassium transporter genes in the peach (*Prunus persica*) using the Hidden Markov model scanning strategy and searching the peach genome database. Utilizing the *Arabidopsis* KT/HAK/KUP family as a reference, phylogenetic analysis indicates that the KT/HAK/KUP family in the peach can be classified into 3 groups. Genomic localization indicated that 16 KT/HAK/KUP family genes were well distributed on 7 scaffolds. Gene structure analysis showed that the KT/HAK/KUP family genes have 6-9 introns. In addition, all of the KT/HAK/KUP family members were hydrophobic proteins; they exhibited similar secondary structure patterns and homologous tertiary structures. Putative *cis*-elements involved in abiotic stress adaption, Ca^{2+} response, light and circadian rhythm regulation, and seed development were observed in the promoters of the KT/HAK/KUP family genes. Subcellular localization prediction indicated that the KT/HAK/KUP members were mainly

located in the plasma membrane. Expression levels of the KT/HAK/KUP family genes were much higher in the fruit and flower than those in the other 7 tissues examined, indicating that the KT/HAK/KUP family genes may have important roles in K^+ uptake and transport, which mainly contribute to flower formation and fruit development in the peach.

Key words: *Prunus persica*; Potassium transporter; KT/HAK/KUP family; Genome-wide analysis

INTRODUCTION

As one of the most important cation elements in plants, potassium (K^+) has key roles in improving plant growth and development (Lebaudy et al., 2007), flower formation (Yamasaki and Yano, 2009), and fruit quality and yield (Demiral and Köseoglu, 2005; Hartz et al., 2005; Nava et al., 2007). In particular, K^+ also contributes to many physiological and metabolic processes in plants (Véry and Sentenac, 2003; Grabov, 2007) that further improve plant tolerance to various abiotic stresses, including drought (Li et al., 2011; Song and Su, 2013), salt (Mian et al., 2011; Upadhyay et al., 2012), and cold (Rai et al., 2008; Ramalho et al., 2013).

K^+ transporters are famous for K^+ acquisition, catalyzing K^+ uptake across a wide spectrum of external concentrations, mediating K^+ movement within the plant and its efflux into the environment, and possible functions in plant cation homeostasis (Véry and Sentenac, 2003; Grabov, 2007). According to the largest K^+ transporter family, KT/HAK/KUP, individual members have been found in evolutionarily diverse organisms (reviewed in Grabov, 2007), including *Arabidopsis*, rice, barley, pepper, tomato, alligatorweed (Song and Su, 2013), and so forth. Several members of the KT/HAK/KUP family have been characterized in plants, either by overexpression in *Arabidopsis* suspension cells (Fu and Luan, 1998) or analyses of T-DNA insertion mutants (Rigas et al., 2001; Mian et al., 2011).

The peach (*Prunus persica*) is among the most economically important fruit crops and grown worldwide. With the help of an available genome database, the peach has become one of the most genetically well-characterized species of the Rosaceae family (Layne and Bassi, 2008; Jung et al., 2008). Current estimates indicate that the peach is a diploid with $N = 8$ chromosomes and has a comparatively small genome, estimated at ~220-230 Mbp, that contains 28,689 transcripts and 27,852 genes, which provide unexpected genetic evidence for further research of *Prunus* spp and other species in the Rosaceae (Jung et al., 2008).

Currently, the use of molecular-assisted selection in conjunction with conventional breeding techniques is an accepted strategy for breeding new peach cultivars with enhanced biotic and abiotic stress resistance, nutritional value, and other novel traits (Layne and Bassi, 2008). In plants, genes of the KT/HAK/KUP family members play significant roles in K^+ uptake and transport related to plant growth and development, as well as various plant biotic and abiotic stress responses (Li et al., 2011; Mian et al., 2011; Upadhyay et al., 2012; Song and Su, 2013). However, a comprehensive analysis of the KT/HAK/KUP family genes in the peach is lacking.

In this study, we initially identified and characterized 16 putative KT/HAK/KUP family genes *in silico* from the peach genome database. According to phylogenetic and protein motif structural analyses, the KT/HAK/KUP family members of the peach were classified into 3 groups, using KT/HAK/KUP members of *Arabidopsis* as a reference. In addition, the

expression percentages of the KT/HAK/KUP family genes in different tissues or organs were analyzed. The data presented in this study may contribute to the selection of excellent candidate genes from the KT/HAK/KUP family in the peach for further functional characterization at the molecular level and elucidate its precise role in improving plant development and stress responses at the physiological level.

MATERIAL AND METHODS

Isolation and localization of predicted KT/HAK/KUP genes in the peach

Protein sequences of KT/HAK/KUP family genes of *Arabidopsis* (Rubio et al., 2000) were obtained from the Phytozome *Arabidopsis* genome database (<http://www.phytozome.net>). These sequences were used as a query to BLAST the peach genome and identify putative homologues in the peach. Nucleic acid and amino acid sequences of the identified putative KT/HAK/KUP members in the peach were obtained from the Phytozome peach genome database. To confirm these predicted KT/HAK/KUP family proteins, the protein sequences were then searched for KT/HAK/KUP domains by using the InterProScan 4.8 (<http://www.ebi.ac.uk/Tools/pfa/iprscan/>) and CD search (<http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>) web servers. The numbers, names, and locations of the conserved domains contained in each KT/HAK/KUP protein sequence of the peach were also recorded.

Motif display and phylogenetic analysis of predicted KT/HAK/KUP genes in the peach

To search the conserved motifs shared by the KT/HAK/KUP proteins, we utilized the online tool of MEME (v4.8.1) (<http://meme.nbcrl.net/meme/cgi-bin/meme.cgi>) via uploading the putative peach KT/HAK/KUP amino acid sequences according to the method described by Bailey et al. (2006). 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>), and a multiple alignment analysis between the peach and *Arabidopsis* KT/HAK/KUP proteins was carried out using the ClustalW program built into the MEGA 4.1 software (Tamura et al., 2007). A phylogenetic tree was constructed by using the MEGA4.0 software between candidate KT/HAK/KUP proteins in the peach and formerly identified members in *Arabidopsis* (Tamura et al., 2007). The KT/HAK/KUP family members in the peach were classified into 3 groups by using a combination of phylogenetic trees and conserved domain analyses of the characteristics and structures of genes.

Gene structure and characteristic analysis of predicted KT/HAK/KUP genes in the peach

In silico approaches were used to obtain the genomic sequences and complementary DNA (cDNA) sequences of the identified KT/HAK/KUP family, and the exon and intron statuses of the KT/HAK/KUP family genes were analyzed using the Gene Structure Display Server software (<http://gsds.cbi.pku.edu.cn/index.php>). In addition, the genomic sequences (peach v1.0), identification (ID) number, gene distribution on scaffold, and genome location of the peach KT/HAK/KUP family genes were obtained from the Phytozome peach genome database.

Characteristics of predicted KT/HAK/KUP proteins in the peach

Assessing the physical and chemical properties of the KT/HAK/KUP proteins with the help of ProtParam online (<http://expasy.org/tools/protparam.html>) (Wilkins et al., 1999) and related indexes, including theoretical isoelectric point (pI), molecular weight, formula, aliphatic index, instability index, and grand average of hydropathicity (GRAVY). Prediction of the KT/HAK/KUP protein secondary structure was carried out via HNN Secondary Structure Prediction (http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_hnn.html), and transmembrane domains were analyzed by the TMPredict online program (http://ch.embnet.org/software/TMPRED_form.html) (Hofmann and Stoffel, 1993). PSORT was used for sub-cellular localization prediction (Emanuelsson et al., 2007). Tertiary structure analysis of the KT/HAK/KUP proteins was conducted using the SWISS-MODEL online server (http://swiss-model.expasy.org/workspace/index.php?func=modelling_simple1) (Benkert et al., 2011), taking the annotated transmembrane transporter protein (PDB ID: 3OB6_B GI:326327903) from *Escherichia coli* as the homologous target template.

Promoter region analysis of predicted KT/HAK/KUP genes in the peach

To investigate *cis*-elements in promoter sequences of the KT/HAK/KUP family genes, 2 kb individual genomic DNA sequences upstream of the initiation codon (ATG) was retrieved from the Phytozome grape genome database (<http://www.phytozome.net>). Then, PLACE (<http://www.dna.affrc.go.jp/PLACE/>) was used to analyze *cis*-elements in the promoters according to the method described by Higo et al. (1999).

Gene expression profiles of predicted KT/HAK/KUP genes in the peach

Expressed sequence tag (EST) data of the peach KT/HAK/KUP members were obtained from the dbEST database on National Center for Biotechnology Information (NCBI; <http://www.ncbi.nlm.nih.gov/dbEST/>) via BLASTN alignment between the corresponding full-length coding sequence (CDS) of the candidate KT/HAK/KUP genes and the NCBI EST database. The corresponding KT/HAK/KUP family ESTs that were characterized with a matching rate >90%, longer than 120 bp, and $E \leq 10^{-10}$ were retrieved and obtained. EST results were classified according to the different tissues and organs, and the expression profiles of the KT/HAK/KUP family genes were investigated.

RESULTS

Identification of predicted KT/HAK/KUP genes in the peach

To identify the KT/HAK/KUP family genes in the peach, BLAST were performed in the peach genome database by using the KT/HAK/KUP proteins of *Arabidopsis* as query sequences. Fortunately, 16 genes were identified as putative KT/HAK/KUP family members in the peach (Table 1). InterProScan results indicated that 16 genes were predicted to encode proteins containing a single KT/HAK/KUP domain of K⁺ transporters (data not shown).

Phylogenetic analysis and motif display of predicted KT/HAK/KUP proteins in the peach

To confirm the obtained classifications and analyze phylogenetic relationships, a phylogenetic tree was constructed based on the alignment of the KT/HAK/KUP amino acid sequences of the 16 and 13 KT/HAK/KUP family members in the peach and *Arabidopsis*, respectively. As shown in Figure 1, the KT/HAK/KUP family members of the peach were classified into 3 groups, including 2, 6, and 8 members using the KT/HAK/KUP members of *Arabidopsis* as a reference. Groups II and III were further divided into 2 subgroups each. It is worth mentioning that ppa025447m of the peach strictly clustered with ApKUP1 of *Arabidopsis* in Group I. Similarly, ppa001638m, ppa001724m, ppa003114m, ppa001652m, and ppa022284m in Group II exhibited close clustering with ApKUP2, ApKUP6, ApKUP8, ApKUP3, and ApKUP4 of *Arabidopsis*, respectively. Moreover, ppa001335m, ppa001373m, and ppa016662m in Group III exhibited close clustering with ApKUP7, ApKUP12, and ApKUP9 of *Arabidopsis*, respectively (Figure 1). Given the above classifications, the 16 KT/HAK/KUP family genes were subjected to further analyses.

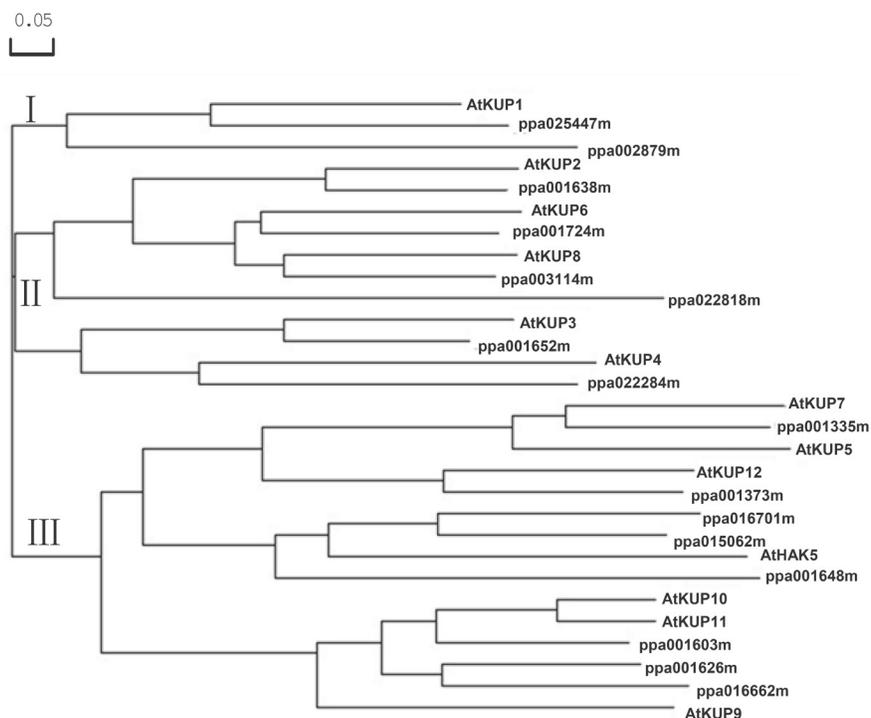


Figure 1. Phylogenetic tree of the KT/HAK/KUP family proteins between the peach and *Arabidopsis*. The tree was created by the bootstrap option of the CLUSTALW multiple alignment packages and the neighbor-joining method using 16 members in the peach and 13 members in the *Arabidopsis* KT/HAK/KUP amino acid sequences. Scale indicates genetic distance. Accession Nos. of the 13 KUP/KT/HAK family members in *Arabidopsis* are as follows: 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, NP_567404.1.

Motif distribution analysis indicated that the KT/HAK/KUP family proteins in the peach possessed 5 discrepant motifs (Figure 2). In particular, Motif 2 had the longest sequence (i.e., 115 amino acids), followed by Motifs 1 and 5; Motif 4 was the shortest, with 39 amino acids (Table 1). The height of a block in Motif 5 indicated the significance of the match with taller blocks. In detail, Motifs 1 and 2 were present in all of the KT/HAK/KUP proteins. Motif 3 was present in all of the KT/HAK/KUP proteins, except for ppa022818m; Motif 4 was present in all of the KT/HAK/KUP proteins, with the exception of ppa002879m. It should be noted that only ppa001652m had Motif 5, implying that ppa001652m may be functionally different from that of the others (Figure 2).

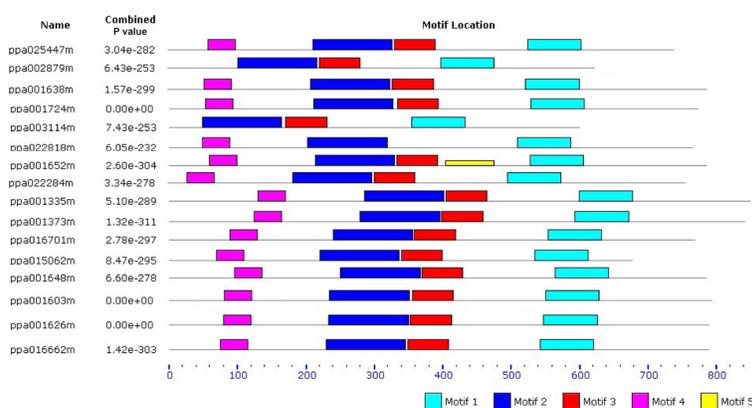


Figure 2. Motif distribution of KT/HAK/KUP family proteins in the peach. Motifs of the KT/HAK/KUP family proteins were investigated on the MEME web server. The green, blue, red, purple, and yellow boxes represent Motifs 1-5, respectively. The length of the gray line shows the length of a sequence relative to all other sequences. The height of a block gives an indication of the significance of the match; taller indicate greater significance.

Table 1. Widths and sequences of the motifs of the KT/HAK/KUP family members in the peach.

Motif name	Width (aa)	Sequence
Motif 1	77	VRVPGIGLVYSELANGVPRIFSHFITNLPANHVSIVFVVCVKYLPVCTVPEEERFLVKRIGPKNFHMFRCVARYGYKD
Motif 2	115	QRHGTDKVGWLFAPVLLWFLVIGGIGMFNIWKYDRSILKAFSPVYVYRFFKRGKGDGWTSLGGIMLSITGTEALFADLSHFVPSIQIAFTSVVFPCLLLAYCGQAAYLMKNSN
Motif 3	59	AFYHSIPDSIYWPVFIVATAA AVVASQATITATFSLIKQALALGCFPRVKV VHTSRKYR
Motif 4	39	IGDPEDLIGALS VIIYSLTLIPLLYV FIVCRANDNGQG
Motif 5	70	RALSPHYIVKFFRETGKDGWISLGGILLSITGTEAMFADLGHFTALSIRLAFAFIIPCLVQYMGQAAF

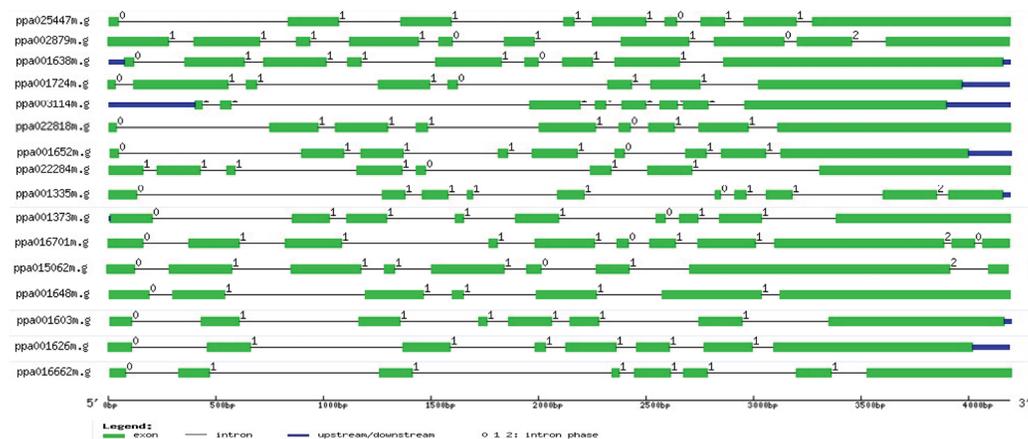
Characteristics of predicted KT/HAK/KUP family genes in the peach

The 16 KT/HAK/KUP family genes were unevenly distributed on scaffolds 1-8 of the peach genome, with the exception of scaffold 7. The numbers of KT/HAK/KUP family genes distributed on scaffolds 1-8 were 4, 1, 2, 4, 2, 1, 0, and 2, respectively (Table 2). Gene ID, gene location, and ORF length are shown in Table 2.

In addition, gene structure analysis showed that the KT/HAK/KUP genes possessed 6-9 introns and varied distinctly in length. In particular, ppa003114m had the largest intron (i.e., >1 kb) and the shortest intron (i.e., <50 bp). In addition, ppa001648m possessed the lowest number of introns (i.e., 6; Figure 3 and Table 2).

Table 2. *In silico* information of the KT/HAK/KUP family members in the peach.

Gene ID	Gene location	Scaffold distribution	ORF (bp)	Intron No.
ppa025447m	1409976-1414335	6	2223	8
ppa002879m	7632757-7636172	4	1878	9
ppa001638m	23561534-23565096	2	2367	8
ppa001724m	25664525-25669164	1	2325	7
ppa003114m	20337885-20342578	3	1880	7
ppa022818m	17899514-17903700	5	2298	8
ppa001652m	21853551-21858762	3	2361	8
ppa022284m	11497124-11502310	5	2277	7
ppa001335m	9561957-9570877	8	2253	9
ppa001373m	25652971-25658468	1	2259	8
ppa016701m	21540564-21544519	1	2310	8
ppa015062m	21560568-21563771	1	2304	7
ppa001648m	21113601-21117430	8	2361	6
ppa001603m	5423542-5428912	4	2385	7
ppa001626m	5406436-5411151	4	2373	7
ppa016662m	5413485-5420065	4	2370	7

**Figure 3.** Gene structure of the KT/HAK/KUP family in the peach. Exons and introns are depicted by filled green boxes and single lines, respectively. Intron phases 0, 1, and 2 are indicated by numbers 0, 1, and 2, respectively, in the figure. Conserved sequences of the KT/HAK/KUP proteins are marked in red. UTRs are displayed by thick blue lines at the 2 ends.

Characteristics of the predicted KT/HAK/KUP family proteins in the peach

The primary structure of the peach KT/HAK/KUP family proteins was calculated from the online ProtParam tool. As shown in Table 3, the shortest and longest amino acid sequences of the peach KT/HAK/KUP family were 601 and 850, respectively, among the 16 members. The GRAVY index indicated that all of the KT/HAK/KUP family members in the peach are hydrophobic proteins. The aliphatic index analyses showed that all of the 16 members had high values (i.e., >100), which also supports the predication that the peach KT/HAK/KUP proteins are hydrophobic proteins. Instability index assays implicated that 10 of the 16 peach KT/HAK/KUP proteins were stable proteins, whereas the remaining 6 members were unstable proteins. In particular, 5 of

the 6 unstable proteins belonged to Group II, including ppa001724m, ppa003114m, ppa022284m, ppa001335m, and ppa001373m; the remaining unstable protein belonged to Group III (i.e., ppa016662m).

In addition, the ProtParam results revealed that the number of amino acids was positively correlated with the molecular weight of the KT/HAK/KUP family proteins. According to the value of theoretical pI, 11 of the 16 KT/HAK/KUP proteins were alkaline amino acids, and the remaining 5 members were acidic amino acids (Table 3). Among the 3 groups, each group contained both alkaline and acidic amino acids.

Table 3. Physical and chemical properties of the KT/HAK/KUP family proteins in the peach.

Gene ID	No. of amino acids	Theoretical pI	Molecular weight (Da)	Formula	Aliphatic index	Instability index	GRAVY
ppa025447m	740	6.88	82380.7	$C_{3785}H_{5904}N_{936}O_{1030}S_{43}$	107.81	35.32	0.455
ppa002879m	625	9.17	69705.6	$C_{3211}H_{5021}N_{813}O_{834}S_{42}$	107.26	33.99	0.479
ppa001638m	788	7.55	88218.0	$C_{4056}H_{6301}N_{1029}O_{1099}S_{36}$	108.72	41.28	0.376
ppa001724m	774	8.61	86847.3	$C_{3975}H_{6202}N_{1018}O_{1088}S_{37}$	103.46	42.95	0.26
ppa003114m	601	7.94	67692.3	$C_{3119}H_{4847}N_{787}O_{835}S_{30}$	107.30	44.26	0.372
ppa022818m	765	7.28	84814.4	$C_{3877}H_{6065}N_{977}O_{1063}S_{45}$	107.02	39.79	0.393
ppa001652m	786	8.68	87534.7	$C_{4057}H_{6304}N_{1030}O_{1080}S_{32}$	111.83	26.45	0.291
ppa022284m	765	7.28	84814.4	$C_{3877}H_{6065}N_{977}O_{1063}S_{45}$	107.02	39.79	0.393
ppa001335m	850	5.29	94701.4	$C_{4322}H_{6793}N_{1081}O_{1218}S_{41}$	109.61	40.05	0.332
ppa001373m	842	6.81	93483.4	$C_{4283}H_{6698}N_{1072}O_{1179}S_{44}$	105.19	43.58	0.386
ppa016701m	769	8.66	85905.3	$C_{3954}H_{6191}N_{997}O_{1082}S_{28}$	106.68	26.45	0.291
ppa015062m	677	8.4	75547.7	$C_{3484}H_{5461}N_{855}O_{940}S_{32}$	109.81	34.67	0.418
ppa001648m	786	6.59	88746.2	$C_{4076}H_{6341}N_{1033}O_{1123}S_{30}$	105.75	32.12	0.185
ppa001603m	794	8.48	88684.6	$C_{4076}H_{6343}N_{1033}O_{1107}S_{36}$	107.49	37.59	0.34
ppa001626m	790	8.45	87966.0	$C_{4038}H_{6305}N_{1025}O_{1094}S_{39}$	108.52	39.26	0.381
ppa016662m	789	6.18	87642.2	$C_{4036}H_{6236}N_{1004}O_{1102}S_{37}$	110.13	43.47	0.471

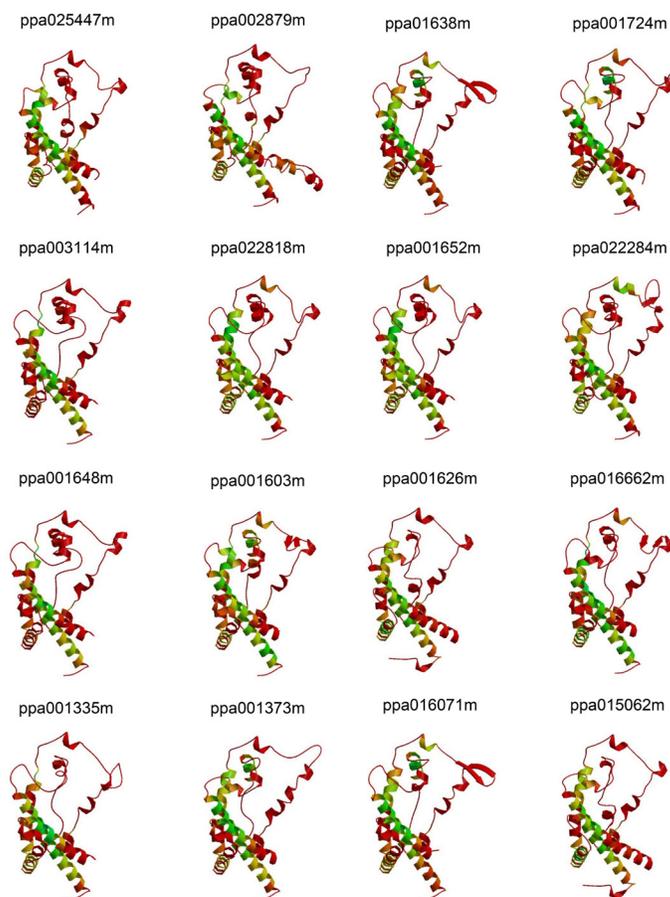
pI = isoelectric point; GRAVY = grand average of hydropathicity.

Moreover, transmembrane (TM) prediction suggested that the KT/HAK/KUP family proteins possessed 12-14 TM domains, which is similar to previously reported KT/HAK/KUP transporters in higher plants (Véry and Sentenac, 2003). Interestingly, ppa022818m and ppa001335m had an N-terminus outside the cell membrane, while the others had an N-terminus inside the cell membrane (Table 4). It is worth mentioning that secondary structure simulations showed that all KT/HAK/KUP family proteins possessed 3 similar patterns (i.e., most prevalent alpha helix, less random coil, and least extended strand; Table 4).

A similar tertiary structure may reflect a corresponding and similar biological function. The tertiary structure of the KT/HAK/KUP family proteins was predicted via the SWISS-MODEL online server. Seemingly, all of the 16 members in the peach exhibited similar tertiary structures (Figure 4), indicating that the KT/HAK/KUP family genes may play homogeneous roles in the biological processes of K^+ uptake and transport; however, further molecular evidence is required for clarification.

Table 4. Secondary structure simulations of the KT/HAK/KUP family proteins in the peach.

Gene ID	No. of transmembrane regions	Secondary structure (%)		
		Alpha helix	Extended strand	Random coil
ppa025447m	13 (N-terminus inside)	51.08	17.16	31.76
ppa002879m	12 (N-terminus inside)	54.24	11.84	33.92
ppa001638m	14 (N-terminus inside)	46.19	18.15	35.66
ppa001724m	13 (N-terminus inside)	44.96	16.54	38.50
ppa003114m	12 (N-terminus inside)	47.75	18.30	33.94
ppa022818m	14 (N-terminus outside)	45.88	20.52	33.59
ppa001652m	14 (N-terminus inside)	43.89	19.08	37.02
ppa022284m	14 (N-terminus inside)	47.36	17.68	34.96
ppa001335m	12 (N-terminus outside)	50.94	14.94	34.12
ppa001373m	12 (N-terminus inside)	54.51	10.81	34.68
ppa016701m	13 (N-terminus inside)	51.24	15.60	33.16
ppa015062m	12 (N-terminus inside)	48.30	15.66	36.04
ppa001648m	13 (N-terminus inside)	50.51	12.09	37.40
ppa001603m	14 (N-terminus inside)	48.74	15.87	35.39
ppa001626m	14 (N-terminus inside)	49.37	15.57	35.06
ppa016662m	14 (N-terminus inside)	43.22	19.52	27.26

**Figure 4.** Tertiary structure prediction of the KT/HAK/KUP family proteins in the peach.

Cis-element analysis

Promoter regions of the KT/HAK/KUP family genes were obtained from the Phytozome grape genome database via retrieving 2-kp range genomic DNA sequences upstream of the translation start sites of the KT/HAK/KUP family genes. By searching the PLACE database, 58 putative *cis*-elements >8 bp in length were identified (data not shown). In particular, many of these *cis*-elements were involved in the abiotic stress response, Ca²⁺-response, light and circadian rhythm regulations, and seed development, including ABRERATCAL (Ca²⁺-responsive), ACGTATERD1 (dehydration responsive), ANAERO1CONSENSUS (anaerobic responsive), BOXLCOREDPCAL (wound responsive), CIACADIANLELHC (circadian regulation), DPBFCORED CDC3 (protein storage), INRNTPSADB (light regulation), PRECONSCRHSP70A (dehydration responsive), RYREPEATGMGY2 (protein storage), SEBFCONSSTPR10A (dehydration responsive), SEF4MOTIFGM7S (protein storage), and TLEGUMINBOX (protein storage).

Subcellular localization and expression profiles of KT/HAK/KUP family proteins in the peach

Presumably, all KT/HAK/KUP family members were mainly localized in the plasma membrane, Golgi body, and endoplasmic reticulum membrane. In addition, 6, 5, and 2 members were also present in the microbody, nucleus, and endoplasmic reticulum lumen, respectively. In particular, ppa001335 was also detected in the chloroplast thylakoid membrane and ppa001626 was also detected in the mitochondrial inner membrane, respectively (Table 5). Seemingly, 7 members, including ppa025447m, ppa002879m, ppa001638m, ppa022818m, ppa016701m, ppa015062m, and ppa001648m, had similar subcellular localization patterns with similar levels. Five members (i.e., ppa001724m, ppa001652m, ppa001373m, ppa001603m, and ppa016662m) possessed identical localization patterns and levels. Ppa003114m and ppa022284m also had similar localization statuses (Table 5). These findings imply that the peach KT/HAK/KUP family members have extensive and delicate localizations in plant cells.

Table 5. Subcellular localization of the KT/HAK/KUP family proteins in the peach.

Gene ID	Plasma membrane	Golgi body	Nucleus	Mitochondrial inner membrane	Endoplasmic reticulum (membrane)	Endoplasmic reticulum (lumen)	Chloroplast thylakoid membrane	Microbody
ppa025447m	0.80	0.40			0.30			0.30
ppa002879m	0.64	0.40			0.30			0.30
ppa001638m	0.80	0.40			0.30			0.30
ppa001724m	0.80	0.40	0.24		0.30			
ppa003114m	0.64	0.46			0.37	0.1		
ppa022818m	0.80	0.40			0.30			0.30
ppa001652m	0.80	0.40	0.24		0.30			
ppa022284m	0.82	0.46			0.68	0.1		
ppa001335m	0.80	0.40			0.30		0.69	
ppa001373m	0.80	0.40	0.24		0.30			
ppa016701m	0.80	0.40			0.30			0.30
ppa015062m	0.80	0.40			0.30			0.30
ppa001648m	0.80	0.40			0.30			0.30
ppa001603m	0.80	0.40	0.24		0.30			
ppa001626m	0.80	0.40		0.42	0.30			
ppa016662m	0.80	0.40	0.24		0.30			

EST data provide valuable information for gene expression research. *In silico* expression profile analyses showed that all of the KT/HAK/KUP family members had corresponding ESTs, except ppa016701m and ppa025447m, with varied sequence numbers. In total, 94 KT/HAK/KUP ESTs were searched in the peach NCBI EST database. Expression profile analyses showed that the predicted KT/HAK/KUP family genes were expressed in different organs or tissues in the peach. The percentages of predicted KT/HAK/KUP family genes expressed in the fruit and leaf were the highest among all of the tissues and organs, respectively (i.e., 30.2 and 22.7%, respectively); they were followed by the flower (12.8%), root (8.8%), flower bud (8.6%), stem (7.5%), embryo (4.3%), seed (3.1%), and xylem (2.0%) (Figure 5).

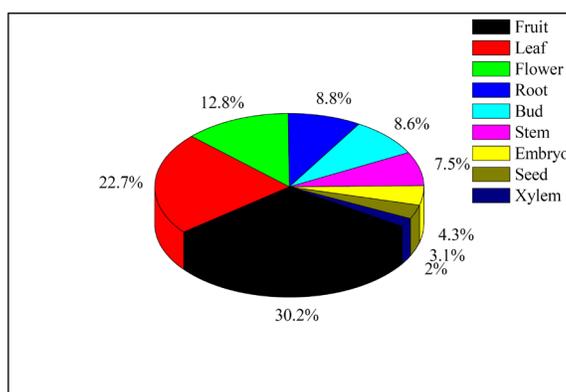


Figure 5. *In silico* expression analysis of the peach KT/HAK/KUP family genes in the different tissues/organs.

DISCUSSION

The ubiquitous presence of these KT/HAK/KUP genes in plants implies that they play important roles in acquiring nutrients and improving plant tolerance to adverse environmental conditions, including drought and K^+ deficiency (Véry and Sentenac, 2003; Grabov, 2007; Song and Su, 2013). Under adverse environments, there is an urgency for plants to strengthen K^+ uptake and transport by plant K^+ transporters. In fruit trees, K^+ especially contributes to flower formation and fruit quality and yield. The extensive tissue/organ-specific expression profiles of the grapevine KT/HAK/KUP family genes imply tremendous K^+ accumulation capacities, which have been indicated for the grapevine genes *VvKUP1* and *VvKUP2* (Davies et al., 2006). Therefore, we conducted *in silico* analysis of the KT/HAK/KUP family genes in the peach genome database to provide a molecular basis for future physiological and molecular studies in the peach.

In the present study, a comprehensive analysis of the genes encoding the KT/HAK/KUP proteins in the peach genome was carried out, resulting in the identification of 16 KT/HAK/KUP family genes. The availability of the complete genome sequences and the previous identification of KT/HAK/KUP family genes from some plant species (Rubio et al., 2000; Bañuelos et al., 2002; Gupta et al., 2008; Song et al., 2011; Zhang et al., 2012) enables the comparison of individual groups, which was based on the classification in *Arabidopsis* as a

criterion. Overall, there were 16 KT/HAK/KUP family genes in the peach, more than in *Arabidopsis* (13) and less than in the grapevine (18), maize (27), and rice (27) (Gupta et al., 2008; Song et al., 2011; Zhang et al., 2012), which may reflect the fact that the peach has a larger genome of 220-230 Mbp (Jung et al., 2008) greater than that of *Arabidopsis* (i.e., 145 Mbp) (Huala et al., 2001) but shorter than that of the grapevine (i.e., 475 Mbp) (Velasco et al., 2007), rice (i.e., 430 Mbp) (Goff et al., 2002), and maize (i.e., 2.3 Gbp) (Schnable et al., 2009).

MEME is widely used to analyze similarities among DNA or proteins sequences, thus producing a motif for each pattern that is discovered. Generally, all the peach KT/HAK/KUP family genes had Motifs 1 and 2, and none of the 5 motif sequences were repeated. Proteins within a group that shared these motifs were likely to share similar functions or have common and recent evolutionary origins. Although ppa025447m and ppa002879m are Group I members, ppa002879m did not have Motif 4, indicating that ppa002879m may possess a distinct function in comparison to the other members. In contrast, ppa002879m and ppa002879m from Group II possessed identical motifs (i.e., 1-3), which might possess similar functions. In particular, the height of a block in Motif 5, which was present only in ppa001652m, gives an indication of the significance of the match with taller blocks; thus, ppa001652m might be functionally different from the other members. Nonetheless, the functions of these 5 motifs of the KT/HAK/KUP family in the peach have not been identified.

Information on the primary amino acid sequences determined the higher structure of a protein (Anfinsen, 1973). Regular secondary structures include the α -helix, β -sheet, random coil, and extended strand. Elements of the secondary structure and super-secondary structure may then combine to form the full three-dimensional-fold of a protein or its tertiary structure (Singh, 2001). Although the primary structure varied among the KT/HAK/KUP family proteins in the peach, their secondary structures exhibited similar patterns, including the most α -helix, less random coils, and the least extended strand (Table 4). Correspondingly, their tertiary structures were also homologous (Figure 4). To a certain extent, similarities in the secondary and tertiary structures of the KT/HAK/KUP family might imply similar and closely related functions in the peach that are still unknown.

Frankly, *cis*-element studies could provide a key foundation for further functional research of the KT/HAK/KUP gene family in the peach. In this study, a series of putative *cis*-elements were found that are involved in the abiotic stress response, Ca^{2+} -response, light and circadian rhythm regulations, and seed development. Such findings are similar to previously reported KT/HAK/KUP members in maize (Zhang et al., 2012). Among these putative *cis*-elements, the water-stress responsive element ACGTATERD1 was distributed in all the 16 KT/HAK/KUP family genes, implying that KT/HAK/KUP family genes in the peach are prone to regulation by drought. Moreover, there were other types of abiotic stress responsive *cis*-elements (i.e., dehydration, anaerobic, and wound responsive). In particular, a widely distributed *cis*-element, ABRERATCAL (a famous Ca^{2+} -responsive element), was detected in 11 of the 16 KT/HAK/KUP family gene promoters. As a secondary messenger, Ca^{2+} responded to several environmental stresses and could activate or deactivate a gene by regulating its *cis*-elements in plants (Gupta et al., 2008). Nonetheless, *cis*-element studies in this article might contribute to further functional regulation research on the KT/HAK/KUP gene family in the peach.

Recently, a great many peach ESTs have been submitted to GenBank by partially sequencing the randomly isolated gene transcripts, which provided valuable resources at the molecular level (Clifton and Mitreva, 2009). In this study, analyses of the expression profiles

of the KT/HAK/KUP family genes showed 9 tissue types, according to the annotation of the ESTs (Figure 5). The percentage of expression of the KT/HAK/KUP family genes was the highest (~30%) in the fruit than the other 8 tissue types. The percentages of the KT/HAK/KUP family genes expressed in the leaf and flower were the second and third among tissues, reaching 22.7 and 12.8%, respectively. Therefore, we speculate that the KT/HAK/KUP family genes may play key roles in K⁺ uptake, and the accumulated K⁺ may be further transported to the aboveground parts that mainly contribute to flower formation and fruit development in the peach. Again, our findings may favorably support the proposition that K⁺ contributes to flower formation, and fruit quality and yield.

In conclusion, the present study shows evidence for the molecular basis of the KT/HAK/KUP family in the peach. Phylogenetic and comparative analyses of the peach KT/HAK/KUP family genes contribute to the identification of excellent genes for detailed characterization and provide useful genetic information for the breeding of new cultivars that may be able to adapt to unfavorable environmental conditions. Currently, electrophysiological and heterogeneous expression studies are underway to explore the molecular mechanisms of the KT/HAK/KUP family, with the intent of providing a direct link between K⁺ accumulation in relation to flower formation, fruit development, and stress-resistance capabilities in the peach.

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