Genome-wide Identification, Phylogenetic and Expression Analysis of ABC1K Gene Family in Tomato (Solanum lycopersicum L.)

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Abstract: Activity of bc1 complex (ABC1K) is a protein kinase commonly found in eukaryotes and prokaryotes. It plays an important role in various developmental and physiological processes, especially critical for plant response to diverse biotic and abiotic stresses. In this study, a genome analysis was carried out and 18 genes of ABC1K family were identified in tomato. Phylogenetic results showed that these members could be classified into three groups – ancestral clade, mitochondrial clade and photosynthetic-specific clade, with several subgroups based on subcellular location prediction by WoLF PSORT and all the SlABC1K proteins contained an ABC1K conserved kinase motifs-VAIK (VAVK, VAMK) and DFG. Conserved motifs analyzed by MEME program indicated that all ABC1K protein contains motif 2, 5, 6 and 8. Predictably, the SlABC1K proteins were localized in chloroplasts or mitochondria; in our analysis of expression patterns, SlABC1K genes could be detected in all tomato organs, and eight genes were specifically expressed in tomato leaf, which implied that the SlABC1Ks might be involved in energy metabolism in tomato. The expression of several genes was significantly changed under abiotic stress, implying their probability of performing various roles in abiotic stresses (NaCl, high temperature, cold, abscisic acid and salicylic acid).

Keywords: Tomato, ABC1K family, Phylogeny, Abiotic stress, Gene function.

Introduction
The ABC1K is an evolutionarily ancient gene family, conserved throughout species of archaea, bacteria and eukaryotes [1-3]. The ABC1K family has also been described as a new family of putative kinases [4]. Unlike typical protein kinases, the ABC1K proteins lack sequence similarity to the ePK domain HMM profile, but have the most conserved kinase motifs, including the VAIK catalytic motif (VAVK and VAMK) with protein kinase activity in Arabidopsis thaliana [3, 5]. The family was first discovered in Saccharomyces cerevisiae [2], the yeast ABC1 gene was located in nucleus, and regulated the correct folding of cytochrome b and assembly of BC1 complex in mitochondrial respiration chain [6, 7]. Recent studies revealed that aarF in Providencia stuartii, a homologue of ABC1K gene (yigR) from Escherichia coli, was required for ubiquinone synthesis and their mutations lead to respiratory defects in bacteria and mitochondria [1, 8]. When an ABC1-like protein from Arabidopsis
expressed in *S. cerevisiae*, it partially repaired the activity of complex III [9]. In human, a homologue of the ABC1K proteins (CABC1) had been identified and possibly involved in apoptosis [10]. ABC1K in plants have more diverse functions than those in yeast [5, 11-17].

Since then, ABC1K genes have been identified, isolated and characterized in many plants, including *Arabidopsis thaliana* [5, 18], rice [18-20], maize [21], wheat [11, 17] and populus [22]. Previous studies showed that, ABC1K protein in higher plants were located in plastids or mitochondria by mass spectrometry and subcellular localization analysis [3, 23, 24]. For example, the Arabidopsis chloroplast protein AtOSA1 (*At5g64940*) was identified as a factor playing a role in the balance of oxidative stress [5]. Another chloroplast protein in *Arabidopsis*, AtACDO1 (ABC1K1, *At4g31390*), plays important roles in mediating chlorophyll degradation and maintaining the number of chlorophyll binding photosynthetic thylakoid membranes, as well as in the photo-oxidative stress response [12]. Recent studies showed that, ABC1K1 (*At4g31390*) kinase constitutes a new type of regulatory link between photosynthetic activity and chloroplast metabolism [16], and ABC1K1/3 (ABC1K3, *At1g79600*) complex contributes to PG function in prenyl-lipid metabolism, stress response, and thylakoid remodeling [25]. In addition, AtSIA1 (*At5g07700*) and AtOSA1 affect iron distribution within the chloroplast and act in signaling pathways that influence responses to ROS production and oxidative stress [13, 14]. Moreover, *AtSIAK* (*At5g24970*) genes involved in stress response of salt [26]. In rice, OsABC1K2 (*LOC_Os02g36570*) play potential roles in regulating dark-induced senescence [27], and OsABC1K8 (*LOC_Os06g48770*) was a negative regulator in response to dehydration stress through ABA-dependent pathway [28]. Meanwhile, OsAGSW1 (*LOC_Os05g25840*), which localized in chloroplasts, plays an important role in seed shape and size by regulating the development of vascular bundles of flag leaves [29]. In addition, ZmABC1-10 (a homology of AtOSA1 in maize) is a cadmium responsive factor and play potential roles in the plant adaption to diverse abiotic stresses [21]. *TaABC1*, a homology of AtOSA1 in wheat, was localized to the cell membrane, cytoplasm, and nucleus, and *TaABC1* overexpression in *Arabidopsis* enhanced drought, salt, and cold stress tolerance, implies that *TaABC1* may act as a regulatory factor involved in a multiple stress response pathways [11]. Another study indicated that characterization of *TaABC1* expression revealed that gene expression was tissue-specific and could be up-regulated by *Puccinia striiformis f. sp. tritici* (Pst) and/or by an abiotic stress like wounding. High-fold induction suggesting that *TaABC1* is a rust-pathotype specific HR-mediator, and down-regulating suggesting *TaABC1* was involved in HR against stripe rust, but overall host resistance is not HR-dependent [17].

*Tomato* (*Solanum lycopersicum*) is the second most consumed vegetable and has been adopted as an important model plant for fruit development [30]. Although the roles of ABC1K genes in development and response to stimuli have been substantially elucidated in *Arabidopsis* and other plants, there is little information concerning the function of ABC1K genes in tomato. In this study, 18 ABC1K genes were identified in tomato genome. The further analysis was carried out to reveal these sequences evolutionary origin, chromosomal location, amino acid sequence conserved motifs, and their expression pattern was determined in different organ and the abiotic stress condition by quantitative real-time PCR analysis. These results will be useful in studies on the function of each gene in the ABC1K families.
Materials and methods

Identification of genes and chromosomal localization

ABC1K members in tomato genome were identified by Arabidopsis. In order to find out tomato ABC1K genes, 17 protein sequences of the Arabidopsis ABC1K members were searched among Solanaceae Genomics Network database (SGN, www.sgn.cornell.edu) [30]. All the obtained sequences were sorted for the unique sequences. ABC1K domain search for these sequences were carried out using Pfam (http://www.sanger.ac.uk/Software/Pfam/search.shtml) [31] and SMART database (http://smart.embl-heidelberg.de/) [32]. In total, 18 ABC1K genes were obtained and named as Solanum lycopersicum ABC1K (SlABC1K) genes. Genes were assigned numbers from SlABC1K1 to SlABC1K18 based on their position from the top to the bottom on the tomato chromosomes 1-12. WoLF PSORT (http://wolfpsort.org/) was used to determine the predicted location of SlABC1K [33].

Phylogenetic analysis

A phylogenetic analysis was performed by aligning all the ABC1K protein sequences of dicots tomato (Solanum lycopersicum, Sl), and poplar (Populus trichocarpa, Ptr) [22], Arabidopsis (Arabidopsis thaliana, At) [5, 18], and monocots rice (Oryza sativa, Os) [18-20] and maize (Zea mays, Zm) [21] with Clustal X 2.0 [34] and an un-rooted neighbor-joining phylogenetic tree was constructed. Bootstrap analysis was carried out taking 1,000 replicates. The MEGA5.1 software was used to view the phylogenetic tree [35]. Similarly, second phylogenetic tree was constructed using ABC1K protein sequences from tomato.

Identification of conserved motifs

Protein motifs of the ABC1K protein sequences were identified statistically using MEME program (http://meme.nbcr.net/meme/) with motif length set as 20-197, motif sites 2-18, maximum number of motifs to find was set at 18, searching given strand only and the distribution of one single motif was any number of repetitions [36].

Digital gene expression analysis

The expression profile was determined through analyzing the RNA-seq data based on locus gene name [37]. The RNA-seq data’s were downloaded from SGN (www.sgn.cornell.edu) [30], including the sequenced data of various tissues in tomato cultivar Heniz 1706.

Plant material and chemical treatment

Tomato seeds (Heinz 1706) were sterilized, rinsed in sterile water and sown in recipient. Plants were grown under standard greenhouse conditions; the culture chamber rooms are set as follows: 14-h-day/10-h-night cycle, 25/20 °C day/night temperature, 80% hygrometry, 250 mmol/m²/s intense luminosity. When fourth leaf appeared the plants were subjected to stress treatment. The stress treatment involved exposure of plants in 1/2 MS liquid medium supplemented with 100 μM/L ABA, 5 mM/L SA or 200 mM/L NaCl respectively and exposed to high temperature at 42 °C in the lighted incubator or cold chamber at 4 °C. 0, 1, 2 and 8 hours were sampled. And also in the vegetative stage to take root, stem and leaf, in the reproductive growth stage take flowers and ripe fruit. The plant tissues were frozen in liquid nitrogen quickly and kept at -80 °C until RNA isolation.

Extraction of total RNA and cDNA synthesis

Total RNA was extracted from tomato organ: roots, stem, leaves, and flowers. Fruit and treatment sample for three biological repeats using a TRIZOL Reagent (Invitrogen) according
to the manufacturer’s instruction. Total RNA was treated by DNase I to remove any genomic DNA contamination and checked by RNA gel. M-MLV Reverse Transcriptase reverses transcription kit synthesized single-stranded cDNA. Then the single-stranded cDNA can be used directly for quantitative real time PCR. Three biological replicates were taken and for each three technical replicates were employed.

Quantitative real time PCR

The reactions were carried out in 96-well optical reaction plates (Applied Bio-systems, USA). Q-PCR was performed using the ABI Prism 7000 Sequence Detection System and Software (PE Applied Bio-systems, USA) [38]. To normalize sample variance, SIUBI3 gene (Solyco1g056940.2.1) was used as the endogenous control. The reaction mixture contained 1 μL cDNA, 0.4 μL PCR primer, 5 μL SYBR and 3.2 μL dd H2O. The PCR ran for 39 cycles at 95 °C for 5 s and 55 °C for 20 s for annealing and extension. The gene specific primers for real-time PCR were designed by primer 3.0 [39] and listed in Table 1. Q-PCR was performed on three biological replicates. After the reaction, the fluorescence curve and melting curve were used to analysis.

Table 1. Primers used for qRT-PCR

<table>
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<th>Sequence</th>
<th>Primer name</th>
<th>Sequence</th>
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<tr>
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Results

The identification of ABC1K genes in tomato

Comprehensive identification of the SlABC1K gene family members in the tomato was achieved using all ABC1K proteins previously reported from Arabidopsis and other plant species [3] in BLAST queries on SGN database. A total of 18 non-redundant SlABC1K genes were identified and manually verified their uniqueness by removing redundant sequences from the databases (Table 2). Since there was no standard annotation assigned to these newly identified genes, we named these SlABC1K genes as SlABC1K1 to SlABC1K18 based on the distribution on the chromosomes (chromosome 1, 2, 3, 4, 6, 7, 8, 9, 10).

The further analysis indicated that the intron number of each gene in tomato was from 0 to 20, open reading frame length ranging from 1428 to 2871, deduced peptide length from 476 to 957, and the isoelectric point from 5.27-9.9 kDa. Previous data showed that the ABC1 proteins were involved in the electron transfer in respiratory chain and located in mitochondria [6]. To understand the subcellular localization of ABC1Ks, WoLF PSORT was used to determine the predicted localization in plant cell. Interestingly, most of the ABC1Ks were located in mitochondria or chloroplast using this programs (Table 2).
Table 2. Basic information for ABC1K family genes in tomato

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<th>Location</th>
<th>Introns</th>
<th>CDS length</th>
<th>Amino acid</th>
<th>pI</th>
<th>Mol.wt.(kDa)</th>
<th>Localization</th>
<th>Chromosome</th>
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<td>67.87</td>
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Chlo: chloroplast; Cyto: cytoplasmic; Mito: mitochondrial; Plas: plasma membrane

Mapping SlABC1K genes on chromosomes

Results indicated that SlABC1K genes were distributed in almost all chromosomes with the exception of 5, 11 and 12 (Fig. 1). Further analysis found that two genes in different chromosomes had homology, such as SlABC1K2 and SlABC1K3, SlABC1K6 and SlABC1K11, SlABC1K5 and SlABC1K12, SlABC1K8 and SlABC1K9, and SlABC1K10. And there were five SlABC1K genes in chromosome 4, three in chromosome 3. The other chromosome (chromosome 1, 2, 6, 9, 10) contains only one gene.

Phylogenetic analysis of ABC1K genes in tomato and other plants

In order to examine the phylogenetic relationship among ABC1 proteins from dicots and monocots plants, a multiple sequences alignment of dicots tomato (Solanum lycopersicum, Sl), poplar (Populus trichocarpa, Ptr) [22], Arabidopsis (Arabidopsis thaliana, At) [5, 18], and monocots rice (Oryza sativa, Os) [18-20] and maize (Zea mays, Zm) [21] was constructed with Clustal X 2.0 and an unrooted tree was built using MEGA5 by employing the Neighbor-Joining (NJ). As shown in Fig. 2, this phylogram was classified into three groups (I, II and III), and based on the primary amino acid sequence, the ABC1 members in groups II and groups III can be further clustered into three (IIa-e) and seven (IIIa-g) subgroups respectively, consistent with the Lundquist [3].

Phylogenetic tree combining gene family from different species will help us to understand the phylogenetic relationships among the members and allows speculation on the putative functions of the proteins based on the functional clades identified. Owe to the functions of several ABC1K proteins have been well characterized experimentally, phylogenetic analysis...
of ABC1K proteins has identified in three clade (Fig. 2), the subgroup I with 12 members from five plants was the ancestral group of ABC1Ks, the second clade consists of the subfamilies IIa-c, which are all likely targeted to the mitochondria based on localization predictions; the subgroup III comprises seven subfamilies (IIIa-g) and is specific for photosynthetic based on the studies of AtABC1K12 (ABC1K1), AtABC1K5 (ABC1K3), AtABC1K17 (AtOSA1), AtABC1K8 (AtSIA1), AtABC1K12 (AtACDO1) and OsABC1K7 (OsAGSW1), which subcellular localization were on chloroplast and involved in chloroplast metabolism, was the photosynthetic-specific clade.

![Fig. 1 Chromosomal distribution and segmental duplication events of tomato ABC1 genes](image1)

![Fig. 2 Phylogenetic tree of ABC1K in tomato and other plants](image2)
Multiple sequence alignment and motifs analyses of SlABC1K proteins

Sequence alignment of the deduced ABC1 proteins in tomato by Clustal 2.1 and MEGA5.1, the result showed that all SlABC1K had a conserved ABC1 domain, and contained VAVK motif and DFG motif (Fig. 3A). Data revealed that ABC1K in tomato is very diverse and putative motifs were predicted by the program MEME and 14 distinct motifs were identified (Table 2). The schematic distributions of the 14 motifs among the different gene groups are presented in Fig. 3B. The most widely distributed motif 2, 5, 6 and 8 were found in SlABC1K1 through SlABC1K18. The motif 11 and 12 were distributed only in two ABC1K protein sequences. The length of conserved amino acid sequence motif within the range was from 21 to 197.

As expected, most of the closely related members in the phylogenetic tree shared common motif compositions, suggesting functional similarities among the ABC1K proteins within the same subfamily, but the unique motifs were shared by different groups. In the same subfamily have similar motifs. Motif 9 existed in III sub-family; motif 1 and motifs 14 exist in I sub-family. We can indicate that the ABC1K motifs were essential for its function.

Fig. 3 Conserved domain and motif analysis of SlABC1K gene

The expression profiles of SlABC1K genes in organ

Since tissue specific transcriptomes of tomato were available for root, leaf, bud, flower and different developmental stages of fruit in SGN database, it was possible to investigate the in-silico expression profiles of ABC1K genes in various tomato tissues. Mapping of the available transcriptome reads revealed expression patterns of 18 SlABC1K genes were retrieved in terms of RPKM values. Hierarchical clustering of the expression profiles showed that several SlABC1K genes exhibited preferential expression in distinct patterns (Fig. 4).
Table 3. Putative conserved motifs in tomato ABC1 proteins predicted by MEME

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Fig. 4 Heat map showing digital expression profiles of SLABC1K genes in various tissues of tomato based on RPLM values

The expression profiles reveal that five genes from subgroup III, such as SLABC1K4, SLABC1K8, SLABC1K10, SLABC1K13 and SLABC1K17 shared similar expression pattern with a high expression levels and especially in flower, leaves, and B+10. And four genes, SLABC1K11, SLABC1K13, SLABC1K15 and SLABC1K18 were specially expressed in root. Some SLABC1K genes were constitutively expressed with low levels in every organ tested, such as those of SLABC1K1, SLABC1K2, SLABC1K3, SLABC1K5 and SLABC1K14 (Fig. 4).

Then, SLABC1K gene expression pattern in different tissues was validation by quantitative real-time PCR (Fig. 5). Most of SLABC1K genes had a high expression levels in leaves, and the leaves relative expression levels of SLABC1K1, SLABC1K7, SLABC1K10, SLABC1K14, SLABC1K17 was over 10. Compared to leaves, their expression levels were lower in roots and flowers. The expression level of SLABC1K2, SLABC1K3, SLABC1K4, SLABC1K5, SLABC1K9, SLABC1K11, SLABC1K13, SLABC1K15 and SLABC1K16 were higher in fruits than in roots and flowers. In summary, ABC1K genes were highly expressed in leave and fruits, then root and stem, finally flower.

The expression profiles of SLABC1K genes under abiotic stress

In order to reveal the relationship between SLABC1K genes and abiotic stresses, the change of gene expression was determined by qRT-PCR under a variety of stress treatment. Tomato
seedlings were subjected to various stresses for 1 h, 2 h and 8 h, basal expression level of all SlABC1K genes was checked in 0 h as one fold, and the expression of all SlABC1K genes was normalized with reference to the expression of SlUBI3 gene. In first 1 h of NaCl treatment (Fig. 6 A,B), the expression of SlABC1K2, SlABC1K3, SlABC1K4, SlABC1K5, SlABC1K6, SlABC1K10, SlABC1K11 and SlABC1K14 were quickly induced, and reach to 2 fold, most of them were keep at the level till the end except SlABC1K2, SlABC1K5 and SlABC1K11. The transcript of SlABC1K9, SlABC1K12, and SlABC1K18 were sharply reduced after NaCl treatment. In other way, the level of SlABC1K1, SlABC1K9, SlABC1K13 and SlABC1K17 were firstly decreased by NaCl treatment, but after 2-hour treatment, their expression returned back (Fig. 6 A,B). Most of the SlABC1K genes were increased expression by cold stress except SlABC1K11 and 12, and had a highest expression levels at 2 h (Fig. 6 C,D). The qRT-PCR results showed that the expression levels of SlABC1K5, SlABC1K12, SlABC1K14, SlABC1K15 and SlABC1K16 were significantly up-regulated after heat treatment, but SlABC1K2, SlABC1K3, SlABC1K7, SlABC1K11, SlABC1K13, SlABC1K17 and SlABC1K18 were down-regulated after heat treatment (Fig. 6 E,F). Among 18 SlABC1K genes, SlABC1K6, SlABC1K7, SlABC1K8, SlABC1K9, SlABC1K10 and SlABC1K12 were up-regulated at 1 h, 2 h and 8 h after ABA treatment, the expression levels of SlABC1K17 have been found to increase by 12.5 folds at 8 h after ABA treatment (Fig. 7 A,B). SlABC1K2, SlABC1K10, SlABC1K11, SlABC1K12, SlABC1K14, SlABC1K15, SlABC1K16 and SlABC1K18 were up-regulated by SA treatment, and SlABC1K14 was increased by 43 folds at 2 h after SA treatment (Fig. 7 C,D).

Fig. 5 Q-PCR analysis of 18 SlABC1K gens in different tissues
**Discussion**

ABC1 (Activity of bc1 complex) is an important protein kinase. Previous studies showed that, ABC1 gene family involved in a wide range of metabolic regulation of plants [3, 6]. Therefore, it is very essential to reveal ABC1K comprehensive significance identification and its function. Compared to 17 members of the ABC1 proteins in *Arabidopsis* [3], 20 in maize [21], 17 in *Oryza sativa* [18-20], 23 in *Populus trichocarpa* [22], we found 18 members of ABC1K protein sequences in tomato genome (Table 2). They were distributed on nine chromosomes (chromosome 1, 2, 3, 4, 6, 7, 8, 9 and 10) (Fig. 1). Previous study showed that, ABC1 proteins have the most conserved kinase motifs, including the VAIK catalytic motif (VAVK and VAMK) [3, 4]. **SlABC1K** protein sequence in tomato had 14 motifs (Fig. 3B); VAVK and DFG are conserved protein sequences in ABC1K protein sequence (Fig. 3A).
ABC1K protein localization and its function have a great relationship. The yeast ABC1K located in the nucleus and mitochondria controlled the correct folding of cytochrome b and the assembly of bc1 complex in the mitochondrial respiration chain [8]. Arabidopsis OSA1 gene, located in chloroplasts, was found to be involved in balancing oxidative stress generated by Cd²⁺, hydrogen peroxide (H₂O₂), and light[5]. In this study, SLABCIK proteins were located in mitochondria or chloroplast through predicted by WoLF PSORT (Table 2). Pervious study shown ABC1K involved in chlorophyll degradation and the response to photooxidative stress in Arabidopsis [12]. In chloroplasts, PGs are formed by blebbing of the outer leaflet of the thylakoid membrane and remain attached to the thylakoid membrane system, providing a conduit for metabolite exchange between the two structures [16, 23, 40]. TaAbc1 and AtOSA1 predominantly expressed in leaves and stems, with only a very low level in roots [5, 17]. Our study found that most SLABCIK genes had a high expression levels in leaves and fruit, and little expression in roots and flowers (Fig. 4 and Fig. 5).

ABC1 genes play an important role in response to abiotic stress and several specific biological processes [3]. Recent studies showed that ABC1K genes in rice leaves could be modulated by a broad range of abiotic factors such as H₂O₂, abscisic acid, low temperature, drought, darkness, and high salinity [19, 20]. The expression of wheat TaABC1L was induced by osmotic, salt, and cold stress and abscisic acid (ABA) treatment, where the peak of expression induced by salt was 30-fold higher than that at the control condition [11]. AtACADO1 RNAi plants were more sensitive to high light and MV-induced photooxidative stress [12]. Our result found that most of SLABCIK genes were induced expression by abiotic stress such as salt, high temperature, cold, ABA and SA (Fig. 6 and Fig. 7), which implies that SLABCIK genes play important roles in response to abiotic stresses.

Conclusion

In conclusion, ABC1K family genes were involved in a wide range of metabolic regulation of plants. It plays an important role in plant growth and development, organ building and hormonal signals. This study provides the genomic framework for further in depth study of the function of SLABCIK in tomato. The expression pattern analysis revealed that SLABCIK genes had a high expression level in leaves, and might have conserved roles in abiotic stress. These results provide a basis for future function of SLABCIK genes.

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