

Genome-wide identification and expression analysis of tomato ADK gene family during development and stress

Lu Yang

Anhui Normal University

Haohao Cao

Chongqing University

Xiaoping Zhang

Anhui Normal University

Liangxian Gui

Anhui Normal University

Qiang Chen

Anhui Normal University

Gui Qian

Anhui Normal University

Jiixin Xiao

Anhui Normal University

Zhengguo Li (✉ zhengguoli@cqu.edu.cn)

Chongqing University

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Abstract

Background: Adenylate kinase (ADK) is widely distributed in organisms and plays an important role in cellular energy homeostasis. In plants, ADK has important functions in plant growth and development regulation as well as adaptation to the environment. However, little information is available about the ADK genes in tomato (*Solanum lycopersicum*), an important economic crop.

Results:

To investigate the characteristics and functions of *ADK* genes in tomato, a total of 11 *ADK* genes were identified and named according to their chromosomal locations. The ADK family in Arabidopsis tomato, potato and rice was divided into six groups and motif analysis revealed that each *SIADK* protein contained five to eight conserved motifs. Sequence analysis revealed 4-19 exons in all *SIADKs* and most members possessed four. Cis-element analysis inferred that several stress response elements were found on the promoters of *SIADKs*. The 11 *SIADKs* were randomly distributed on nine of the 12 tomato chromosomes. Three duplication events were observed between tomato chromosome, and a high degree of conservation of synteny was found between tomato and potato. The online TomExpress platform prediction revealed that *SIADKs* were expressed in various tissues and organs, basically consistent with the data obtained from real-time quantitative PCR (qPCR). The qPCR verification was also used to determine the expression level of *SIADKs* and demonstrated that the genes responded to multiple abiotic stresses, such as drought, salt and cold. Besides, the qPCR results showed that *SIADK* transcription was responsive to most of the applied hormone treatment: methyl jasmonate, ethylene, salicylic acid, indole 3-acetic acid and abscisic acid. Notably, *SIADK2* and *4* exhibited significant changes under multiple stress treatments. Furthermore, correlation networks analysis revealed co-expressed genes between *SIADKs* and other tomato functional genes.

Conclusions: These results provide valuable information for clarifying the evolutionary relationship of the tomato *ADK* family and in aiding functional characterization of *SIADKs* in further research.

Background

The monophosphate of adenosine, also referred to as adenylate (AMP), is one of the four main mononucleotides making up ribonucleic acid in cells. Moreover, the formation of AMP is often accompanied by the release of energy in organisms [1]. Therefore, adenylate metabolism is an important part of primary metabolism and the change in adenylate content is the main factor affecting cell metabolism [2]. There are three important adenylate forms in organisms: AMP, adenosine diphosphate (ADP) and adenosine tri-phosphate (ATP). The ratio of AMP, ADP and ATP determines the energy charge ratio and carbohydrate metabolism, which directly affects plant growth and development as well as the ability to resist stress [3, 4].

Adenylate kinase (ADK, EC 2.7.4.3) is a ubiquitous and abundant enzyme found in virtually all living organisms [5]. It catalyzes a reversible transphosphorylation reaction ($ATP+AMP \leftrightarrow 2ADP$) and is

considered to be a crucial enzyme in maintaining energy metabolism and the pool sizes of various adenylates at equilibrium [6, 7]. Usually, ADKs have three domains: a large central CORE domain, nucleoside monophosphate binding domain and an ATP-binding domain [8, 9]. In plants, ADK has been found in a wide range of species with different subcellular locations, such as cytosol, mitochondria, and plastids, and its enzyme activity demonstrated in the cases of maize, rice, and potato [10-13]. Potato is a solanaceous plants abundant in starch, and suppression of *StADK* expression in potato plastids enhanced content of adenylic acid and significantly improved starch production [12]. *Arabidopsis thaliana* is a good model plant for studying plant growth and development. In Arabidopsis, T-DNA insertion mutants disrupted in one *ADK* gene At2g37250 showed increased amino acid levels and enhanced root growth [13]. Subsequently, another relevant study revealed that disruption of Arabidopsis *ADK* gene At5g47840 leads to loss of chloroplast integrity, causing a bleached phenotype from early embryo to seedling development [14]. Very recently, researchers found that ADK3 in the chloroplasts of a green alga could interact with the chloroplast glyceraldehyde-3-phosphate dehydrogenase to form a stable complex, and the interaction might be a mechanism to regulate the crucial ATP: NADPH ratio in the Calvin-Benson cycle [15].

Besides regulation of growth and development, ADK is also widely involved in abiotic stress responses in plants. When roots and stems of maize were treated with solutions of two different ratios of $\text{Ca}^{2+}/\text{Na}^{+}$, results showed that ADK content had an important relationship with plants salt stress [16]. In tomato, microarray analysis of genes revealed that an *ADK* homolog (SGN-U214214) was repressed in salt-treated tissues [17]. Other microarray data revealed that *ADK* gene (SGN-U232826) expression in drought-tolerant tomato was induced by drought stress [18]. Using pea seeds as a model to research the adenylate balance in dehydrating and imbibing seeds driven by mitochondria, results indicated that ADK played a crucial role in building and later using the huge AMP pool which appears as a signature of the dry state in seeds [19].

Tomato is one of the most important agricultural products worldwide, as well as an important model for studying fleshy fruit development and ripening [20]. Currently, the *ADK* family in tomato has not been studied systematically. Due to the importance of the *ADK* genes in regulating plant growth and stress resistance, it would be of interest to make a systematic investigation of the *ADK* family in tomato. In the present study, we used bioinformatics methods to identify *ADK* genes from the tomato genome, and analyze the phylogenetic relationships, sequence features, gene location, chromosomal locations, evolutionary relationship and cis-elements in promoters. The comprehensive transcriptomic profiling of the *ADK* family in various tissues and organs of tomato during different developmental stages were carried out using the online TomExpress platform. Also, the dynamic expression patterns of the *ADK* family in response to different plant hormones methyl jasmonate (MeJA), ethylene (Eth), salicylic acid (SA), indole 3-acetic acid (IAA) and abscisic acid (ABA) and abiotic stresses (drought, cold and salt stress) were systematically studied in detail using quantitative real-time PCR (qRT-PCR). Combined expression data of *SIADKs* in tomato different developmental stages and stress treatments groups obtained from TomExpress platform, co-expression and correlation networks between *SIADKs* and

other tomato functional genes were further investigated. In brief, the present results will provide useful information for further functional and regulation mechanism investigations of the *ADK* family in tomato.

Methods

Plant materials and growth conditions

Solanum lycopersicum cv. Micro-Tom was used as wild type plant material. The plant samples used in this study were collected from Key Laboratory of Plant Hormones and Development Regulation of Chongqing, School of Life Sciences, Chongqing University, Chongqing, China. Collection of plant materials complied with the institutional, national and international guidelines. No specific permits were required. To assess potential roles of *SIADK* family genes throughout tomato development by experiments, the tissues of roots (R); stems (S); leaves (L); bud (B); flowers (F); immature green fruit (IMG); mature green fruit (MG); breaker stage fruit (BR); orange fruit (O); red fruit (R) and over ripe fruit (OR) were collected from wild type tomato, frozen in liquid nitrogen immediately and stored at -80°C for RNA extraction lately. For stress and hormone treatment, germinated seeds were cultivated in greenhouse with suitable conditions: 16/8 h light/dark cycle, 25/18°C day/night temperature, 80% relative humidity and 250 $\mu\text{mol}/\text{m}^2/\text{s}$ light intensity. The seeds and subsequently growing plants were watered daily. In addition, the plants were irrigated with nutrient solution once per week. One-month old tomato plants with good growth status were selected and transplanted from soil to hydroponic box for hydroponic culture (pH 5.8), and the Hogland nutrient solution was renewed regularly. Hydroponics were adapted for 5-6 days to eliminate the damage caused by transplantation, then abiotic stresses and hormone treatments experiments were performed with these plants.

Identification of tomato *ADK* genes

The *ADK* genes in tomato were comprehensively identified by two methods. The first method is using 'adenylate kinase' as keywords inputted in the annotation text to search unigene families in the SOL Genomics Network (<http://solgenomics.net>) [21]. The second method is screening *ADK* family genes with bio-linux system. First, we downloaded tomato genome information files in Ensembl database (<http://plants.ensembl.org/index.html>), including the gff, cds, pep, fasta suffix files. Then, gunzipping these files and acquiring corresponding relationship between gene and mRNA. Subsequently, the hidden markov model (HMM) file corresponding to the ADK domain (PF00406) was downloaded from Pfam protein family database (<http://pfam.sanger.ac.uk/>) [22]. Hmsearch command was then used to search the *ADK* genes from tomato genome database. The default parameters were adopted and the cutoff value was set to 0.01 [23]. All candidate genes that may contain ADK domain based on HMMER results were further examined by confirming the existence of the ADK core sequences using the Pfam and SMART program [24]. In a word, the tomato *ADK* gene family was identified with two above mentioned methods, then the identified members were further verified combined with gene description in SOL Genomics Network and gene sequence blast in National Center for Biotechnology Information (NCBI) database.

Characteristics, phylogenetic relationship and sequence analysis of ADK proteins

Among other species, twelve *ADK* family genes in potato were also identified according to the above methods. In rice, seven *ADK* family genes has been screened in China Rice Data Center (<http://www.ricedata.cn/gene/>) and RGAP (<http://rice.plantbiology.msu.edu/>). Also, seven adenylate kinase isoforms has been found with high sequence homology in Arabidopsis genome [14]. The full-length *ADK* proteins in Arabidopsis and identified in tomato, potato, and rice were aligned using ClustalW. Phylogenetic analysis of *ADK* proteins was performed using MEGA 7.0.26 with the neighbor-joining (NJ) method based on the Poisson model [25], the bootstrap method was used to test the tree with 1000 replicates, and paired deletion was performed [26]. Multiple sequences alignments of the amino acid sequences of tomato *ADK* proteins were analyzed by ClustalX 1.83. and DNAMAN software. The MEME online program (<http://meme.nbcn.net/meme/intro.html>) provides a unified portal for online discovery and analysis of sequence motifs representing features, such as protein interaction domains [27]. Here the MEME Suite Web server for protein sequence analysis was used to identify conserved motifs in the identified tomato *ADK* proteins, with the number of found motifs as ten and the other parameters as default values. Then sequences of the motifs were blast in Pfam (<http://www.pfam.org/>) and SMART (<http://smart.embl.de/>) database. The *ADK* protein sequences in tomato were analyzed by the online ProtParam tool of ExPASy (<http://web.expasy.org/protparam/>) for physical and chemical characteristics. The prediction parameters included the number of amino acids, molecular weight (MW) and theoretical pI [28]. For predicting subcellular localization of mature proteins, four online tools were also employed including: CELLO (<http://cello.life.nctu.edu.tw/>), Wolf Psort (http://www.genscript.com/psort/wolf_psort.html), Predotar (<https://urgi.versailles.inra.fr/Tools/Predotar>) and TargetP (<http://www.cbs.dtu.dk/services/TargetP/>).

Gene structure, chromosome location and CREs of *SIADK* genes

The visualization of gene structure and annotation features helps to determine the function and evolution of family genes intuitively. Physical locations of all *SIADK* genes on each chromosome were obtained from tomato genome database: SOL Genomics Network (<http://solgenomics.net>). The exon-intron structure of each *SIADK* was determined by aligning the full-length cDNA sequence with the genomic DNA sequence. The Gene Structure Display Server 2.0 (GSDS2.0; <http://gsds.cbi.pku.edu.cn//index.php>) program was used to display the gene structures on the basis of the coding sequences (exons), intron and untranslated regions (UTR) composition information [29]. With the strength of the genome annotation, MapChart software was used for mapping the genomic location and relative distances of *SIADK* genes in the chromosome [30]. The 1500 bp upstream sequences of the *SIADK*-coding sequences were retrieved from the SOL Genomics Network and then submitted to PlantCARE (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>) to identify stress- and hormone- related cis-regulatory elements (CREs) [31].

Synteny analysis of *SIADK* genes

For analysis of inter-chromosomal relationships of *SIADK* genes, the gene duplication landscape was obtained using the MCScanX [32]. Each duplicate segment with *SIADK* genes was selected, and the syntonic map was generated using CIRCOS [33]. The putative duplicated genes were linked by the connection lines. To analysis of synteny relationships of *SIADK* genes between different species, the genome sequences, genome annotation information and ADK coding sequences for rice (<http://www.ricedata.cn/gene/>), tomato (<https://solgenomics.net/>) and potato (<https://solgenomics.net/>) were obtained. Fragment lengths in the alignment that exceeded 75% of the length were confirmed as tandem duplications. The synteny relationships between the *ADK* family member in tomato and these species were determined using MCScanX.

Expression data mining of tomato *SIADK* genes

Expression patterns of identified tomato *SIADK* family genes during vegetative and reproductive development were carried out with TomExpress bioinformatics platform (<http://gbf.toulouse.inra.fr/tomexpress>). TomExpress provides a unified and standard method to judge tomato gene expression from released RNA-Seq data sets. Expression data represent normalized counts per base and mean values of multiple cultivars for each tissue and stage, as well as different biotic and abiotic stress treatment. The expression value is appropriately associated with corresponding experimental annotations. Various forms of data output such as line diagram, heat map and other graphics types have been utilized to make the web pages more user-friendly. For co-expression and correlation networks analysis, co-expression tool of TomExpress platform was used to identify genes that displayed similar or opposite expression profiles, the comprehensive visualization of co-expression results were based upon the calculation of the correlation values [34, 35].

Hormone and abiotic stress treatments

One-month-old tomato plants which have been transferred to hydroponic box from the soil were subjected to hormone and abiotic stress treatments. For hormone treatment,

200 μ M ethephon (Eth), 100 μ M abscisic acid (ABA), 500 μ M salicylic acid (SA) and 50 μ M Methyl Jasmonate (MeJA) have been prepared. Then tomato leaves were sprayed with above solution, respectively. When the solution drops, stop spraying and quickly seal the leaves with transparent plastic film for moisture retention. After treatment for 0, 0.5, 1, 2, 3, 6 and 12 h, 24 h, samples of leaves were harvested separately. Plants leaves sprayed with ddH₂O were used as control. For salt stress treatment, salt of NaCl was added into hydroponic medium to ensure the final salt concentration was 150 mM, it

was necessary to submerge root adequately with salt solution. Samples were collected after 0, 3, 6, 9, 12, 24, 48 and 72 h treatment. For cold stress treatment, tomato plants for hydroponic culture were transferred to a cold chamber maintained at 4 ± 1 °C. Leaves were sampled at 0, 3, 6, 9, 12, 24, 48 and 72 h post treatment and untreated plants were used as controls. For drought treatment, PEG6000 was used for simulating drought. The final concentration of PEG6000 in hydroponic medium was 12% and it was necessary to submerge root adequately with PEG6000 solution. Samples were collected after 0, 3, 6, 9, 12, 24, 48 and 72 h treatment. Three individual plants in good status were used for each treatment. After treatment, tissue of leaves in each biological replicate was collected and mixed thoroughly, then frozen in liquid nitrogen immediately and stored at -80°C .

RNA isolation and quantitative real-time PCR analysis

Total RNA was extracted using TRIzol reagent (Invitrogen, USA) according to the manufacturer's instructions. After RNA integrity detection and RNA concentration measurement, *DNase I* (Thermo Scientific) was used to remove any genomic DNA. About 2 µg of total RNA from each sample was used for first-strand cDNA synthesis following the manufacturer's protocol. DNAMAN software was used to design primers specific to the *SIADK* family genes (Table S5) and *SIActin* (Solyc03g078400) was used as internal control. Quantitative real-time PCR was conducted on a CFX96 Touch™ Real-Time PCR Detection System (BIO-RAD, USA) using the SuperReal PreMix Plus (SYBR Green) (TIANGEN, China). Each reaction mixture was 20 µL sample volume in total contained 1.2 µL of cDNA, 1.2 µL of primers mix, 10 µL of 2×SYBR Mix Taq and 7.6 µL of sterile distilled water. The PCR amplification cycle was performed as follows: 95 °C for 15 min, 40 cycles at 95 °C for 10 s, and 60 °C for 30 s. Melting curve analysis was performed ranging 60 to 95 °C to verify amplicon specificity for each primer pairs. Relative expression levels of the *SIADK* genes were calculated using the standard curve and normalized by the control's expression. The results were displayed with heatmap to displaying gene clustering based on expression patterns.

Results

Identification of the *ADK* family in tomato

To identify the *ADK* family in tomato, unigenes were searched in the SOL Genomics Network and a hidden Markov model search was used to scan probable proteins. After confirming the existence of the *ADK* core sequences according to the description in tomato genome database and the Pfam and SMART programs, a total of 11 *ADK* genes were identified. Based on their chromosomal locations, the genes were assigned as *SIADK1-11*. Gene information of each *ADK* gene and physicochemical properties of matched proteins were predicted with tomato gene database and ExPASy online tool, respectively. The names and IDs of genes, chromosomal and strand locations, open reading frame lengths, exon numbers, amino acid numbers, molecular weights (MWs), isoelectric points (pIs) are listed in Table 1. Predicted subcellular location of *SIADK* proteins are listed in Table 2. The coding sequence (CDS) and gene sequence of the

SIADK family are found in Table S1. The lengths of the SIADK proteins ranged from 630 (SIADK1) to 1989 amino acids (SIADK11) and the corresponding range for MWs was 22872.10- 74110.62 Da (Table 1). The 11 *SIADK* genes were distributed on nine tomato chromosomes, with *SIADK3* and 4 located in forward strands and the others in reverse strands. The predicted pI values of SIADKs ranged from 5.76 (SIADK1) to 8.83 (SIADK4). Prediction results of subcellular localization based on four different online tools revealed that most SIADK proteins were presumably located in mitochondria (e.g. SIADK2, 3, 4 and 6); SIADK1 and 5 may located in cytoplasmic; and SIADK8 and 11 may located in chloroplast. Interestingly, SIADK9 may located in cytoplasmic or nuclear, and SIADK10 may located in chloroplast or mitochondria.

Phylogenetic analysis and multiple sequence alignment of *SIADK* genes

The phylogenetic relationship of tomato SIADK proteins, together with seven Arabidopsis AtADKs, twelve potato StADKs and seven rice OsADKs were examined by multiple sequence alignment with full lengths of the amino acid sequences. Then the phylogenetic analysis was conducted by MEGA7 based on the aligned results, and the neighbor-joining method was used with bootstrap replications of 1000. The ADK family was divided into six groups (Fig. 1A). SIADK6 and 10 together with two potato homologs (Sotub09g006620 and Sotub04g013920), two Arabidopsis homologs (AT2g39270 and AT2g37250) and one rice homolog (Os03t0130400) were phylogenetically distinct and formed the predicted first branch of plant ADKs. The second branch contained two isoforms of potato (Sotub02g037180 and Sotub03g005270), one isoforms of Arabidopsis (AT3g01820), two isoforms of rice (Os08t0118900 and Os07t0412400) and two of tomato (SIADK2 and 3). The third branch contained three evolutionarily similar subbranches: SIADK1 and Sotub01g028550; SIADK4 and Sotub03g020180; and SIADK9 and Sotub08g022760. The fourth branch contained two isoforms of potato (Sotub06g024300 and Sotub11g015570), one isoforms of Arabidopsis (At5g47840), one isoforms of rice (Os08t0109300) and one of tomato (SIADK8). The fifth branch contained four subbranches: At5g50370 and At5g63400, SIADK5 and Sotub03g023880; SIADK7 and Sotub05g016010; and Os12t0236400 and Os11t0312220. At last, SIADK11 together with Sotub12g00740, Os08t0288200 and AT5g35170 were formed as branch VI. Multiple sequence alignment of SIADK proteins was performed using ClustalX 1.83 and DNAMAN software (Fig. 1B). Because the amino acid sequence of SIADK11 was much longer than other proteins, the last comparison part which only contained ADK11 C- terminal amino acids is not shown in Fig. 1B.

Motif analysis

According to the amino acid sequences, the MEME web server was used to search the conserved motifs that were shared with the SIADK proteins. A total of 10 distinct conserved motifs were found and the number of the motif residues ranged within 38- 50 amino acids; details of the conserved motifs are shown in Fig. 2A. Each SIADK protein contained five to eight conserved motifs. It is worth noting that motifs 1, 2 and 4 were fundamental in the ADK domains because they were shared by all SIADKs (Fig. 2B). Among the three motifs, motif 2 has been marked in Fig. 1A because the conserved domain belonged to it. As shown in Fig. 2B, some SIADKs (e.g. SIADK1 and 9, SIADK2 and 3, and SIADK5 and 7) shared common motif compositions which supported the grouping results. In addition, motif 7 was

unique to *SIADK5* and *7* at the N-terminal, and motif 9 was unique to *SIADK2* and *3* at the C-terminal. The specific motifs may contribute to the functional divergence of *SIADK* genes. In order to better annotate function of genes, the sequence of each motif has been performed blast in pfam and SMART database, the prediction result showed that except motif 7 to 10 were too short to predict, motif 1 to 6 all contained ADK domain (Table S3).

Gene structure and chromosomal location analysis of *SIADKs*

Gene organization plays a vital role in the evolution of multiple gene families [36]. The neighbor-joining phylogenetic tree constructed with MEGA7 is shown in Fig. 3A, which was consistent with the result in Fig. 1A. Corresponding to each gene, the genomic sequence and cDNA sequence information were submitted to the Gene Structure Display Server together to show the gene structure. Among these genes, the average gene length was 1610-9218 bp. *SIADK 11* had the maximum number of exons and the longest gene length, while *SIADK2* was the shortest genes. Each gene had 4-19 exons and the majority of *SIADKs* harbored four exons (*SIADK2, 3, 6* and *10*) (Fig. 3A, Table 1). The result also revealed that genes closed to each other in phylogenetic tree had similar gene structure, such as *SIADK2* and *3, SIADK6* and *10, SIADK5* and *7, and SIADK1* and *9*. Tomato contained 12 chromosomes, the 11 *SIADKs* were distributed on nine of them randomly (Fig. 3B). The majority of *SIADKs* were located on the proximate or distal ends of the chromosomes. Chromosome 03 had the greatest number of predicted *SIADKs*, with three (*SIADK3-5*), and no *SIADK* existed on chromosomes 07, 10 and 11. Only one *SIADK* existed on each of the other eight chromosomes.

Cis-regulatory elements in *SIADK* promoters

To pave the way for further study of potential gene function and regulatory mechanism of the *SIADK* family, especially during abiotic stress responses and hormone treatment, the 1500-bp upstream sequences from the translation start sites of *SIADKs* were submitted to PlantCARE to detect cis-regulatory elements (CRE). After screening, CRE related to stress and hormone were retained and detailed information could be found in Table S4. Also, the location and number of representative 17 CRE (ABRE, ARE, AuxRE, Box4, CGTCA-motif, G-box, GATA-motif, LTR, MRE, MYB, MYC, P-box, TATC-box, TC-rich, TCA-element, TGA-element, TGACG-motif) were visualized on each gene with GSDS software (Figure 3C). The upstream regulatory sequence of promoters contained multiple elements that respond to hormones (such as Box 4 and G-box, TGACG-motif) and stress signals (such as TC-rich, LTR, MYB- and MYC-binding sites), which indicated that expressions of *SIADKs* were associated with abiotic stresses and hormone signal transduction response. It is worth noting that from the promoter region of -1500 bp to -700 bp, cis-regulatory elements of *SIADK11* and *1* were less distributed and almost nonexistent, respectively.

Synteny analysis of *SIADK* genes

Synteny analysis of *SIADK* genes were conducted to investigate the duplication event occurring in the tomato *ADK* family (Fig. 4A). Three duplication events were observed between chr01 and chr08 (*SIADK1* and *SIADK9*), chr02 and chr03 (*SIADK2* and *SIADK3*), and chr04 and chr09 (*SIADK6* and *SIADK10*), which

evolved from segment duplication. Interestingly, each gene pair existed duplication events belonged to the same subfamily in the phylogenetic tree (Fig. 3A). To further infer the phylogenetic mechanisms of tomato *ADK* gene family, we constructed two comparative syntenic maps of tomato associated with two representative species, rice and potato (Fig. 4B). The homology between tomato and potato are closer because they both belong to solanaceae while rice belongs to gramineae. Our result revealed that ten *SIADK* genes showed syntenic relationship with potato genes (*SIADK1* and *Sotub01g028550*, *SIADK2* and *Sotub02g037180*, *SIADK3* and *Sotub03g005270*, *SIADK5* and *Sotub03g023880*, *SIADK6* and *Sotub04g013920*, *SIADK7* and *Sotub05g016010*, *SIADK8* and *Sotub06g024300*, *SIADK9* and *Sotub08g022760*, *SIADK10* and *Sotub09g006620*, *SIADK11* and *Sotub12g007490*), while no collinear relationship existed of *ADK* genes between rice and tomato.

Expression patterns analysis of *SIADKs*

Comprehensive transcriptomic profiling of 11 *SIADKs* in tomato vegetative and reproductive tissues was carried out using the online TomExpress platform and associated data mining tools (<http://gbf.toulouse.inra.fr/tomexpress>) (Fig. 5A and Fig. S1). Gene *SIADK1* was seldom expressed in all tomato tissues. Genes *SIADK5* and *10* showed higher expression in seed and root than other genes; *SIADK10* showed increased expression in flower and fruit, peaking at bud in 3 mm and stage of mature green fruit (35 DPA). Expression of *SIADK5* was enhanced at the onset of fruit development and reached its peak at mature green fruit (35 DPA); however, during ripening, its expression decreased until increasing again during the red fruit stage. Some genes such as *ADK2* and *6* exhibited relative moderate expression in all tissues. Of particular interest, *SIADK3*, *8* and *9* were the most highly expressed during late fruit ripening, displaying a net up-regulation at the onset of ripening starting after the mature green stage. This pattern of expression suggests a potential role of these genes in regulating the ripening process.

To assess the potential roles of *SIADKs* throughout tomato development, we conducted detailed quantitative real-time PCR (qRT-PCR) to examine transcription in different tissues (Fig. 5B and C). In non-fruit tissues, including root, stem, leaf, bud and flower, *SIADK1* and *4* showed similar expression patterns. Notably, the expression levels of most *SIADKs* was drastically enhanced in bud compared to other tissues and expression of *SIADK2* was much higher in bud. Genes *SIADK2*, *3*, *6*, *7*, *8* and *10* also showed similar expression patterns, possibly indicating redundancy of gene function. Interestingly, *SIADK11* expression was higher in leaves than other tissues. Additionally, expression of *SIADK5* and *9* showed little difference among tissues (Figure 4B). During the critical stages of fruit development, including immature green stage (IMG), mature green stage (MG), breaker stage (BR), orange stage (O), red ripe stage (RR) and overripe stage (OR), the expression levels of *SIADK4*, *7* and *10* were relatively high at BR stage and those of *SIADK3* and *6* were relatively high at O stage. It was remarkable that the mRNA level of *SIADK9* was significantly up-regulated at RR stage, and interestingly, expression of almost all detected genes was relatively low in OR stage. Due to the quite low expression abundance and amplification efficiency of *SIADK1*, *2* and *11*, it was difficult to get satisfactory qPCR results for analysis of gene expression (Fig. 5C).

Expression characteristics of *SIADKs* under diverse abiotic stresses

To identify potential functions of *SIADKs* in response to different abiotic stresses, their transcript profiles were assayed under drought, salt and cold treatments (Fig. 6). Following 3 h of treatment with PEG6000, the expression of most *SIADKs* was obviously up-regulated except for *SIADK1*, 3 and 8. Interestingly, expression of most *SIADKs* decreased rapidly during 9-12 h after treatment, but increased rapidly during 24-48 h. Especially, the transcript levels of *SIADK1* and 11 almost linearly increased with time under PEG6000 treatment during 12-72 h. Under salt treatment, expression levels of most *SIADKs* were obviously down-regulated at the early stage. It should be noted that almost all *SIADKs* contained two expression peaks at 9 and 48 h; however, the two expression peaks for *SIADK7* were at 6 and 24 h. Under cold stress, there was obvious up- and down-regulation in expression levels of *SIADK3* during 12-24 h and 24-48 h, respectively. For *SIADK7*, expression showed no significant differences for all time points before 24 h of treatment, but expression rose rapidly during 24-72 h. Notably, *SIADK7* exhibited unique changes under multiple stress treatments, suggesting that it may have a unique role in stress responsiveness. Moreover, the result of gene clustering showed that homologous genes always had similar expression patterns especially under PEG6000 treatment, such as *SIADK2* and 3, and *SIADK5* and 7 (Fig. 3A and Fig. 6).

Expression profiles of *SIADKs* in response to diverse hormone treatments

Previous evidence indicated that different hormones play important roles in stress signal transduction and cell responses [37-39]. Here, we investigated the expression profiles of *SIADKs* in response to Eth, IAA, ABA, SA and MeJA treatments (Fig. 7). With Eth treatment, in general, the expression of most *SIADKs* showed little difference, but that of *SIADK1*, 2 and 7 increased gradually to different levels during 0-2 h. With IAA treatment, the expression peaks of *SIADK1*, 2 and 4 were at 2, 0.5 and 6 h, respectively. Interestingly, *SIADK11* showed decreased transcription at the early stage and almost no expression at later time points. At 0.5 h after ABA treatment, expression of *SIADK5*, 7 and 10 showed no significant differences; however, expression of *SIADK3*, 4, 6, 8, 9 and 11 decreased, and that of *SIADK1* and 2 increased at the first time point. Moreover, most *SIADKs* did not change significantly after 1-12 h of treatment. *SIADK6* showed decreased transcription at the early stage and almost none at following time points. With SA treatment, transcription of most *SIADKs* was repressed in the first 0.5 h. Expression of most *SIADKs* showed mild change under SA treatment, except for *SIADK2*, 6 and 7. Especially, *SIADK2* and 7 showed similar expression patterns throughout all time points, with peak expression at 12 h. The plant regulator MeJA mediates diverse developmental processes and defense responses. It rapidly induced up-regulation of *SIADK4* and 2 during 1-6 h and 3-12 h time points, respectively. Expression of *SIADK6* increase gradually during 1-6 h, with opposite results for expression of *SIADK9* during 0-3 h. Similar to IAA treatment, *SIADK11* also showed decreased transcription at the early stage and almost no expression for later time points. Hence, transcription of *SIADKs* was responsive to most of the applied stress treatments. Interestingly, *SIADK2* and 4 exhibited significant changes under multiple hormone treatments, suggesting that they may have unique roles in hormone regulation. Notably, the result of gene clustering showed that the homologous genes of *SIADK6* and 10 had similar expression patterns with the treatment of Eth and

IAA; and *SIADK8* and *11* had similar expression patterns with the treatment of Eth and ABA (Fig. 3A and Fig. 7).

SIADKs expression patterns under stress based on RNA-seq data

As introduced above, TomExpress provides a unified and standard method to judge tomato gene expression from released RNA-Seq data sets. Here, *SIADKs* expression patterns under treatment with different plant hormone and multiple hormones were analyzed (Fig. 8 and Fig. S2). In general, the expression of most *SIADKs* (*SIADK3*, *5*, *6*, *7* and *10*) were lower in leaves (C10 to C17) than in roots (C1-C2, C4-C5, C7-C8) whether these tissues were treated with cytokinin or not. Also, the expression of *SIADK1* was lower in many tissues, and even could not be detected in roots (C2, C4, C5, C7) and leaves (C10, C13, C16) under special stress conditions. With the treatment of auxin in tip of roots, the expression of *SIADK7* and *10* were down-regulated significantly. In fruit, several *SIADK* genes such as *SIADK3*, *5*, *8* and *10* displayed higher expression levels. Compared with treatment of ACC in fruit, multiple hormones treatment of ACC+ IAA could up-regulated the expression of *SIADK3* and *5* (Fig. S2). Heatmap can be very convenient to show gene clustering. As shown in Fig. 8, under treatment with different plant hormone and multiple hormones, *SIADK3*, *4*, *5*, *7* and *10* showed similar expression patterns in many tissues, so were the group of *SIADK2*, *6* and *11* and the group of *SIADK1*, *8* and *9* (Fig. 8).

Co-expression and correlation networks analysis.

Based on expression data of *SIADKs* under 44 global conditions of different development and stress treatment derived from TomExpress platform, co-expression and correlation networks were analyzed (Table S6 and Fig. 9). The correlation values of co-expressed genes pairs were calculated and the correlation threshold was set as 0.92. Then, the pairs of co-expressed genes of which the correlation coefficient was more than |0.92| were displayed (Table S6). The results showed that *SIADK5*, *8*, *9* and *11* possessed the number of 17, 3, 4 and 6 co-expressed genes, respectively. Among the 17 genes existed correlation with *SIADK5*, 16 showed positive correlation and 1 showed negative correlation; Among the 3 genes existed correlation with *SIADK8*, 1 showed positive correlation and 2 showed negative correlation. However, the 6 co-expressed genes of *SIADK11* and the 4 co-expressed genes of *SIADK9* showed only positive and negative correlation, respectively. Furthermore, these correlation data were visualized as a heatmap after a hierarchical clustering to highlight the positively and negatively correlated groups. The result showed that *SIADK8* (*Solyc06g065270*) and *9* (*Solyc08g077300*) showed similar co-expression patterns, while *SIADK5* (*Solyc03g111200*) and *11* (*Solyc12g010380*) showed quite different co-expression patterns (Fig. 9).

Discussion

ADK is ubiquitous in the kingdoms Animalia and Plantae and it is found in the cytosol and also in many organelles such as mitochondria and chloroplasts. So far, ADK-encoding genes have been cloned from a wide variety of plant species [13]. However, genome-wide analysis of the *ADK* gene family has not been pursued in tomato, a model plant for studying plant fruit ripening. In the current study, 11 *SIADKs* in

tomato were identified, and designated *SIADK1-11* on the basis of their chromosomal location (Table 1). The phylogeny, motif, gene structure, chromosomal location, cis-elements and expression patterns in different tissues and under stress treatments were analyzed. Synteny analysis was also performed in genome of tomato and among several related plant species. Combined with expression data sets from TomExpress platform, co-expression and correlation networks were investigated between *SIADKs* and other tomato functional genes. This study provides comprehensive information on the *SIADK* family and will aid understanding of the function of *SIADKs*.

Previous research revealed seven *ADK* isoforms with high sequence homology in the Arabidopsis genome [14]. The lower number of *ADK* genes in Arabidopsis may be related to its small genome [40]. Potato belongs to solanaceae and has strong homology with tomato. In this plant, 12 *ADK* genes were identified. And also, rice belongs to gramineae which contains 7 *ADK* genes (Table S2). The *ADK* proteins from the four plant species were classified into six groups, with genes in the same group showing a closer evolutionary relationship. For example, *SIADK6* and 10 belonged to group I; *SIADK2* and 3 belonged to group II; and *SIADK1*, 4 and 9 belonged to group III. It is worth noting that *SIADK1*, 4, and 9 were already identified as being closely related to UMP-CMP kinases, with highest homology to the respective three genes in Arabidopsis. Much closer evolutionary relationships existed in the same subbranch. Interestingly, each *ADK* family member of tomato has a potato *ADK* family member with high homology, such as *SIADK1* with Sotub01g028550, *SIADK2* with Sotub02g037180, and so on (Fig. 1A). Subcellular localization prediction showed that *SIADKs* were distributed in mitochondria, chloroplast and other plastids in cells, with the greatest occurrence in chloroplasts (Table 2). This is consistent with previous reports that *ADK* activity in plants is mainly distributed in the chloroplast matrix and mitochondrial membrane space [41-43].

Motif analysis revealed a total of 10 motifs (Fig. 2A), with motifs 1, 2, and 4 shared by all *SIADKs*. In addition, motif 7 was unique to *SIADK5* and 7 at the N-terminal, and motif 9 was unique to *SIADK2* and 3 at the C-terminal (Fig. 2B). Common motifs imply functional redundancy and the specific motifs may contribute to the functional divergence [44]. For the evolution of multiple gene families, the model of gene organization is very important [36]. Gene structure analysis revealed 4-19 exons in each *SIADK* (Fig. 3A). All above mentioned genes in the same subbranch (Fig. 1A and Fig. 3A), such as *SIADK1* and 9, *SIADK2* and 3, and *SIADK5* and 7 shared common motif compositions (Fig. 2B) and similar gene structure (Fig. 3A). This correlation between gene structure and motif arrangement further confirmed the classifications of the *SIADKs*.

17 CRE related to hormone regulation and stress response were analyzed (Table S4 and Fig. 3C). When plants are exposed to abiotic stresses such as salt, drought or low temperature, ABA-dependent and -independent pathways are simultaneously activated [45, 46]. Genes involved in the ABA-dependent pathway not only induce ABA biosynthesis, but also regulate the expression of genes containing ABREs [47, 48]. The ABREs mainly occurred in *SIADK3* and 6 and the G-box element was mostly distributed in *SIADK3* (Table S4, Fig. 3C). In the barley *HVA22* gene and the *Lea* gene promoter, the core sequence ACGT of G-box and other regulatory sequences (CE1 and CE3) constitute an ABA response complex to facilitate

the transcription strength regulation of ABA-regulated genes [49]. The MYB elements are found in the promoters of several stress-resistance genes in Arabidopsis [50]. Our results showed MYB elements distributed in all *SIADKs*, especially *SIADK2* and *3* (Fig. 3C). The MYC element is a cis-acting element in response to drought and ABA, and exists in a variety of anti-stress gene promoters, with reports related to soybeans and Arabidopsis [51, 52]. Our results revealed that MYC existed in almost all *SIADKs* except *SIADK1* and *7*, and was distributed frequently in *SIADK6* (Fig. 3C).

Gene duplications play important role in the evolution of plant genome and genetic system [53]. Duplicated genes promote the generation of new genes and their corresponding new functions. Three principal evolutionary patterns contain segmental duplication, tandem duplication and transposition events, the former two patterns can often lead to gene family expansion [54, 55]. Our results revealed that in tomato genome, three duplication events were observed between chromosomes, which evolved from segment duplication (Fig. 4A). Compared with other related species, except *SIADK4*, ten other *SIADKs* showed syntenic relationship with potato *StADK* genes (Fig. 4B). It was found that gene pairs existed collinear relationship were also closed to each other in the phylogenetic tree (Fig. 1A and Fig. 4B). These results indicated whole-genome duplication (WGD) event occurred and *SIADK4* may have new gene function.

The expression patterns of *ADK* genes in different tissues have been described in many species, including Arabidopsis [14] and rice [10]. In Arabidopsis, expression of *AtADKs* was detected in leaves, roots, and 16-d-old seedlings and *AtADK1-5* were much more expressed than *AtADK6*, while *AtADK7* was at the detection limit [14]. However, there was no uniform gene expression pattern for *SIADKs* in tomato. Our qPCR results for *SIADKs* in tomato were basically consistent with those predicted by the online TomExpress platform. For example, the prediction expression peak of *SIADK2* and *11* was in bud of 3 mm and leaves, respectively, which was highly consistent with the qPCR result (Fig. 5A, B). Also, it was difficult to get satisfactory qPCR results for gene expression analysis of *SIADK1*, *2* and *11* due to quite low expression abundance and amplification efficiency, which was consistent with the software prediction that these three genes had very low expression levels in fruit (Fig. 5A, C). For qPCR detection, *SIADK2*, *3*, *6*, *7*, *8* and *10* also showed similar expression patterns, which hint at similarities in structures, redundancies in functions as well as shared induction mechanisms (Fig. 5B).

Abiotic stresses, such as drought, high salinity, extreme temperature and flooding are major causes of crop loss worldwide, reducing average yields for most major crop plants by more than 50% [56]. In addition to regulation of growth and development, previous study showed that *ADKs* are widely involved in abiotic stress response in plants [16-19]. In our study, transcript profiles of the tomato *ADK* family were assayed under drought, salt and cold treatments. With the increased time of PEG6000 treatment, expression of most *SIADKs* was up-regulated, especially of *SIADK1* and *11*. Microarray data revealed that the expression of an *ADK* gene (SGN-U232826) in drought-tolerant tomato was induced by drought stress [18]. The sequence of SGN-U232826 was consistent with the *SIADK10* identified in our study, which was induced during 24-72 h following PEG6000 treatment (Fig. 6). The maintenance of mitochondrial ATP synthesis during water stress is essential for preserving plastid function, and increased *ADK* gene

expression may indicate the ability to provide more ATP for maintaining cellular activities under drought stress [18, 57]. With PEG6000 treatment, gene clustering results showed that *SIADK6* and *10*, *SIADK5* and *7*, and *SIADK2* and *3* possessed similar expression patterns which supporting the gene sequence homology. Several enzymes, such as ADK and catalase, were specially induced by drought but repressed under salt stress in tomato [17]. In tomato, microarray analysis of genes revealed that an *ADK* homolog (SGN-U214214), which is the same gene of *SIADK10* named here, was repressed in salt-treated tissues [17]. Also, in the present study, almost all *SIADKs* contained two expression peaks at 9 and 48 h; however, the two expression peaks for *SIADK7* were at 6 and 24 h. With NaCl treatment, gene clustering results showed that *SIADK2* and *3*, *SIADK8* and *11*, possessed similar expression patterns which supporting the gene sequence homology. With cold treatment, *SIADK6* and *10* displayed quite different expression patterns although their sequences were highly homologous. In addition, *SIADK3* and *7* responded strongly to cold treatment, indicating that they may play a role in cold stress. (Fig. 6).

Previous evidence indicated that different hormones play important roles in cell responses and stress signal transduction [37-39]. For example, IAA is involved in almost all aspects of plant growth and development, from embryogenesis to senescence, from root tip to shoot tip [58]. Eth is a key regulator during fleshy fruit ripening [59]. ABA is a crucial phytohormone induced by biotic or abiotic stress, and plays important roles in plant tolerance to abiotic stresses [60]. MeJA play an important role in alleviating biotic (pathogens and insects) and abiotic stresses in plants [61]. Our study showed that the transcripts of these *SIADKs* were responsive to most hormone treatments (Fig. 7 and Fig. 8). In plants, many hormones need to cross function. For example, two plant hormones, ABA and Eth, play an important role in the complex story of abiotic stress and, consequently, cross-talk between these two has been reported [62]. Also, both Eth and SA play important roles in biotic stresses [63]. Notably, *SIADK2* and *4* exhibited significant changes under these hormone treatments, suggesting that they have unique roles in hormone responsiveness (Fig. 7). So, understanding the response of *SIADKs* to hormones can lay a foundation for further elucidating their functions in plant growth and stress response.

Gene co-expression network analysis (GCNA) is a genetic approach for investigating correlations between genes using large-scale gene expression profiling data which is especially useful for finding relationships between phenotypic traits and functional modules [64, 65]. Besides a lot of ribosomal proteins owing high correlation relationship with *SIADK* under 44 global conditions, other proteins such as threonine-protein kinase and fructose-1 6-bisphosphatase also possessed higher correlation with *SIADK* family members. High positive correlation coefficient (0.92) existed between *SIADK8* and one resistance protein (Nbs-Irr), which hinted that *SIADK8* may have potential role in stress resistance (Table S6, Fig. 9).

Conclusions

A total of 11 *ADK* genes were identified here and named according to their chromosomal locations. The phylogeny, motif, gene structure, chromosomal location, cis-elements, evolutionary relationship, co-expression analysis and expression patterns prediction in different tissues were analyzed with bioinformatics method. The qPCR verification results revealed the expression level of *SIADKs* in different

tomato tissues was basically consistent with prediction results. Additionally, the qPCR revealed that the *SIADKs* responded to multiple abiotic stresses and plant hormones. Notably, *SIADK7* exhibited unique changes under multiple stress treatments, and *SIADK2* and *4* exhibited significant changes under multiple plant treatments. Analysis of co-expression and correlation networks between *SIADKs* and other tomato functional supply new idea for exploring gene function. In general, the study provides comprehensive information on the *SIADK* gene family tomato and will aid in determining the *SIADK* gene function in further research.

Abbreviations

MeJA: methyl jasmonate; Eth: ethylene; SA: salicylic acid; IAA: indole 3-acetic acid; ABA: abscisic acid; AMP: monophosphate of adenosine; ADP: adenosine diphosphate; ATP: adenosine tri-phosphate; qRT-PCR: Quantitative real-time polymerase chain reaction; NCBI: National Center for Biotechnology Information; CREs: cis-regulatory elements; AA: Amino acid; ABRE: ABA responsive element; GSDS: Gene structure display server; HMM: Hidden markov model; MW: Molecular weight; ORF: Open reading frame; PI: Isoelectric point; chr: Chromosome; UTR: Untranslated region; IMG: immature green; MG: mature green; BR: breaker; O: orange; RR: red ripe; OR: overripe; dpG: day post growth; DMSO: dimethyl sulfoxide

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

All data generated or analyzed during this study are included in this published article [and its supplementary information files.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

LY collected the public dataset, perform bioinformatics analysis and also drafted the manuscript. HHC and LXG performed the experiments. XPZ and JXX contributed to data collection. QC and GQ contributed to bioinformatics analysis. ZGL conceived this study and reviewed the manuscript. All of the authors read and approved the final manuscript.

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Tables

Table 1 Features of *SIADK* family genes identified in tomato

Name	Gene ID	Chr	Genomic Location	Strand	ORF	Exon	AA	MW(kDa)	PI
SIADK1	Solyc01g088480.2.1	1	83267069-83271080	-	630	10	209	22872.10	5.76
SIADK2	Solyc02g093990.2.1	2	54662319-54663928	-	780	4	259	29003.16	6.45
SIADK3	Solyc03g005050.2.1	3	44287-46883	+	852	4	283	31654.06	6.46
SIADK4	Solyc03g083610.2.1	3	53563661-53567185	+	714	10	237	26768.76	8.83
SIADK5	Solyc03g111200.2.1	3	61838312-61842194	-	735	6	244	26669.91	8.57
SIADK6	Solyc04g049690.2.1	4	43054097-43062321	-	810	4	269	30011.55	6.90
SIADK7	Solyc05g014980.2.1	5	9188640-9193694	-	729	6	242	26541.56	7.01
SIADK8	Solyc06g065270.2.1	6	40705359-40709638	-	861	7	286	31970.68	6.96
SIADK9	Solyc08g077300.2.1	8	61201659-61206762	-	651	8	216	24411.10	7.63
SIADK10	Solyc09g007180.2.1	9	801969-804985	-	846	4	281	30520.98	6.36
SIADK11	Solyc12g010380.2.1	12	3429721-3438938	-	1989	19	662	74110.62	6.67

Chr: chromosome

Due to technical limitations, Table 2 is only available as a download in the supplementary files section.

Figures

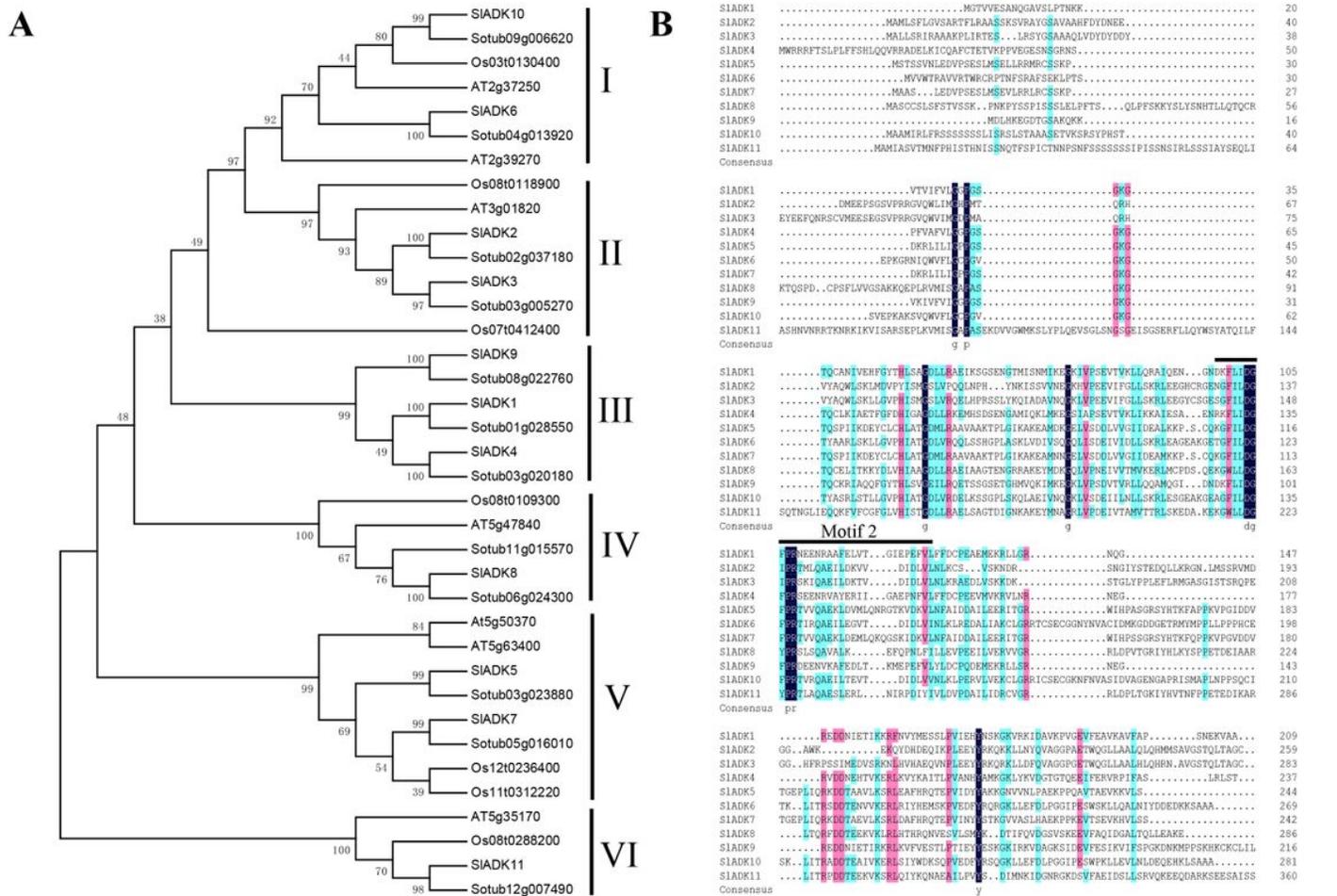


Figure 1

Phylogenetic analysis and multiple sequence alignment of adenylate kinase (ADK). A. Phylogenetic analysis of tomato ADK family members, together with 7 Arabidopsis ADKs, 12 potato ADKs and 7 rice ADKs. Full-length of ADK proteins in these species were aligned using ClustalW and phylogenetic trees was carried out by the neighbour-joining method on MEGA7. Each branch was marked out with Roman numerals. B. Multiple sequence alignment of SIADKs. SIADK proteins were aligned using ClustalX2 program and DNAMAN software. The sequence that matches exactly was marked blue and motif II was marked out as a representative.

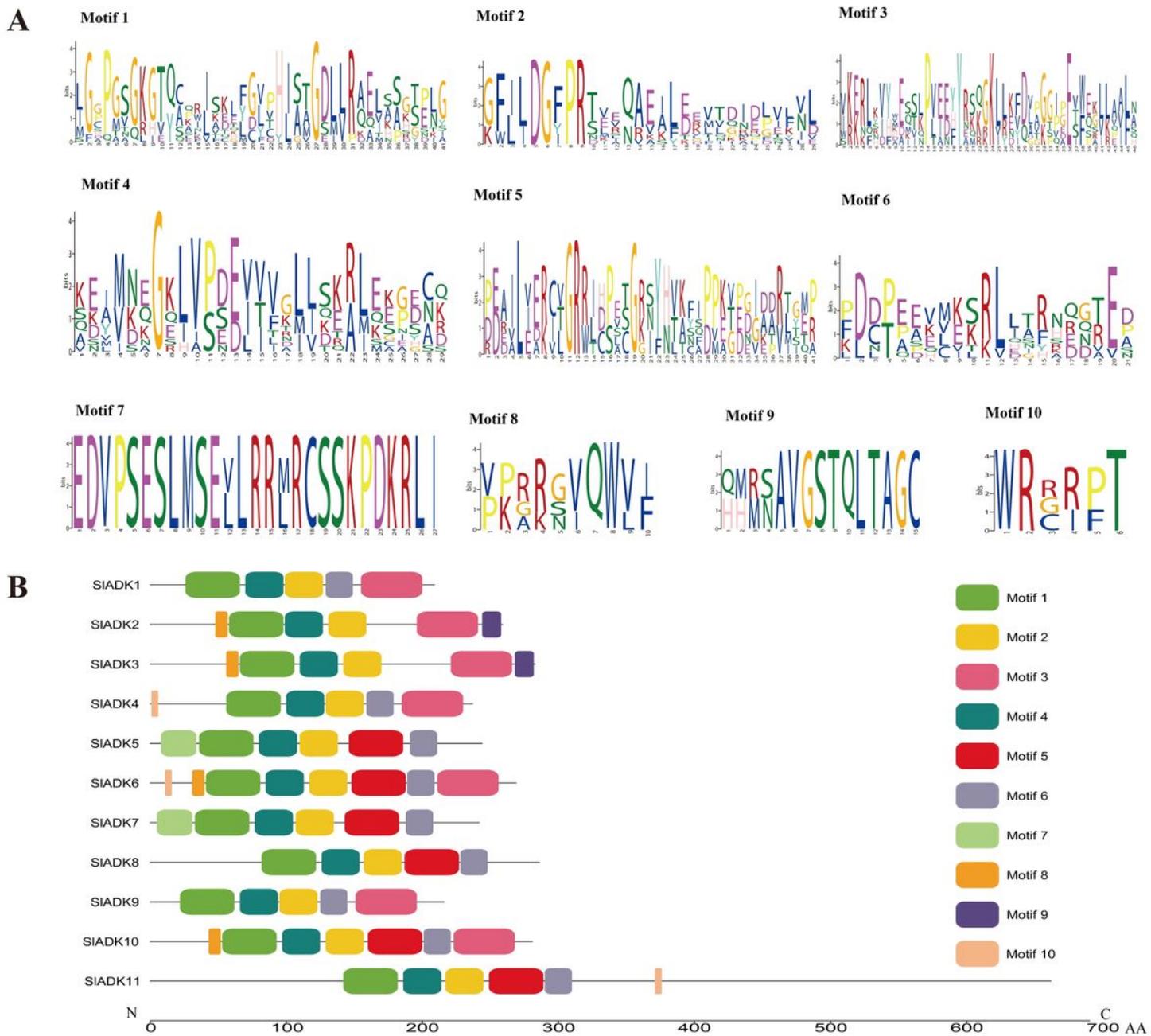


Figure 2

Details of ten conserved motifs and their distributions in SIADK proteins. A. Details of ten selected motifs. Ten conserved motifs in SIADK proteins were generated by the online MEME tool. The overall height of the stack represented the level of sequence conservation. Heights of residues within a stack indicated the frequency of each residue at the indicated position. B. The motif composition of tomato SIADK proteins. Ten putative motifs were indicated in different colored boxes. The length of protein can be estimated using the scale at the bottom.

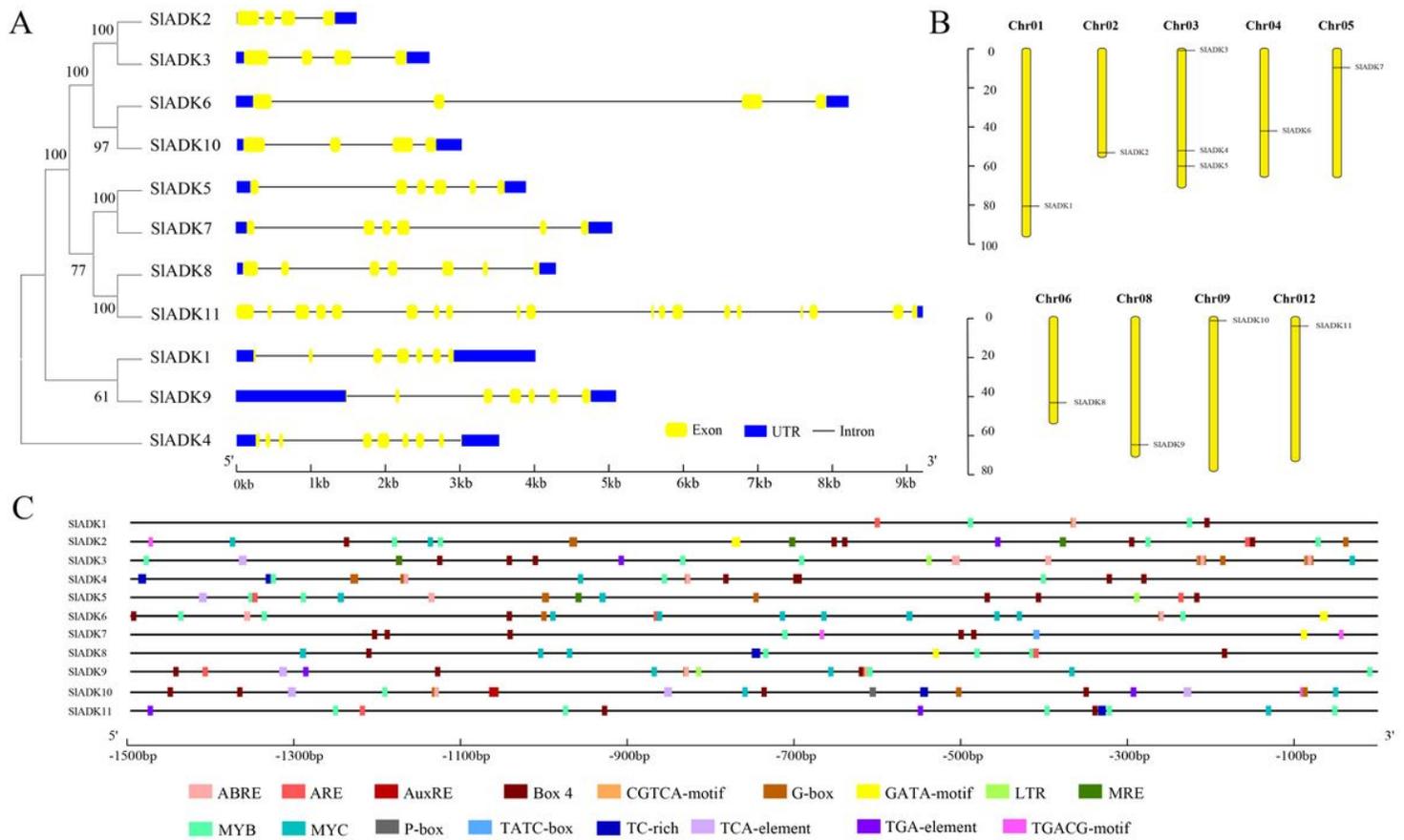


Figure 3

Phylogenetic relationship, gene structure, chromosomal location and predicted cis-elements of SIADK gene family. A. phylogenetic tree (left) of 11 SIADK proteins. The unrooted neighbor-joining phylogenetic tree was constructed with MEGA7 using full-length amino acid sequences of 11 SIADK proteins, and the bootstrap test replicate was set as 1000 times. Exon/intron organization (right) of SIADK genes. Yellow boxes represent exons and black lines with same length represent introns. The UTR region of SIADK genes are indicated in blue boxes. The length of exons can be inferred by the scale at the bottom. B. Chromosomal location of SIADK genes. The number of chromosomes was indicated at the top of each chromosome. The scale on the left is in megabases (Mb). C. Predicted stress- and hormone-related cis-elements in SIADK promoters. Promoter sequences (-1500 bp) of 11 SIADK genes are analyzed by PlantCARE. The upstream length to the translation start site can be inferred according to the scale at the bottom.

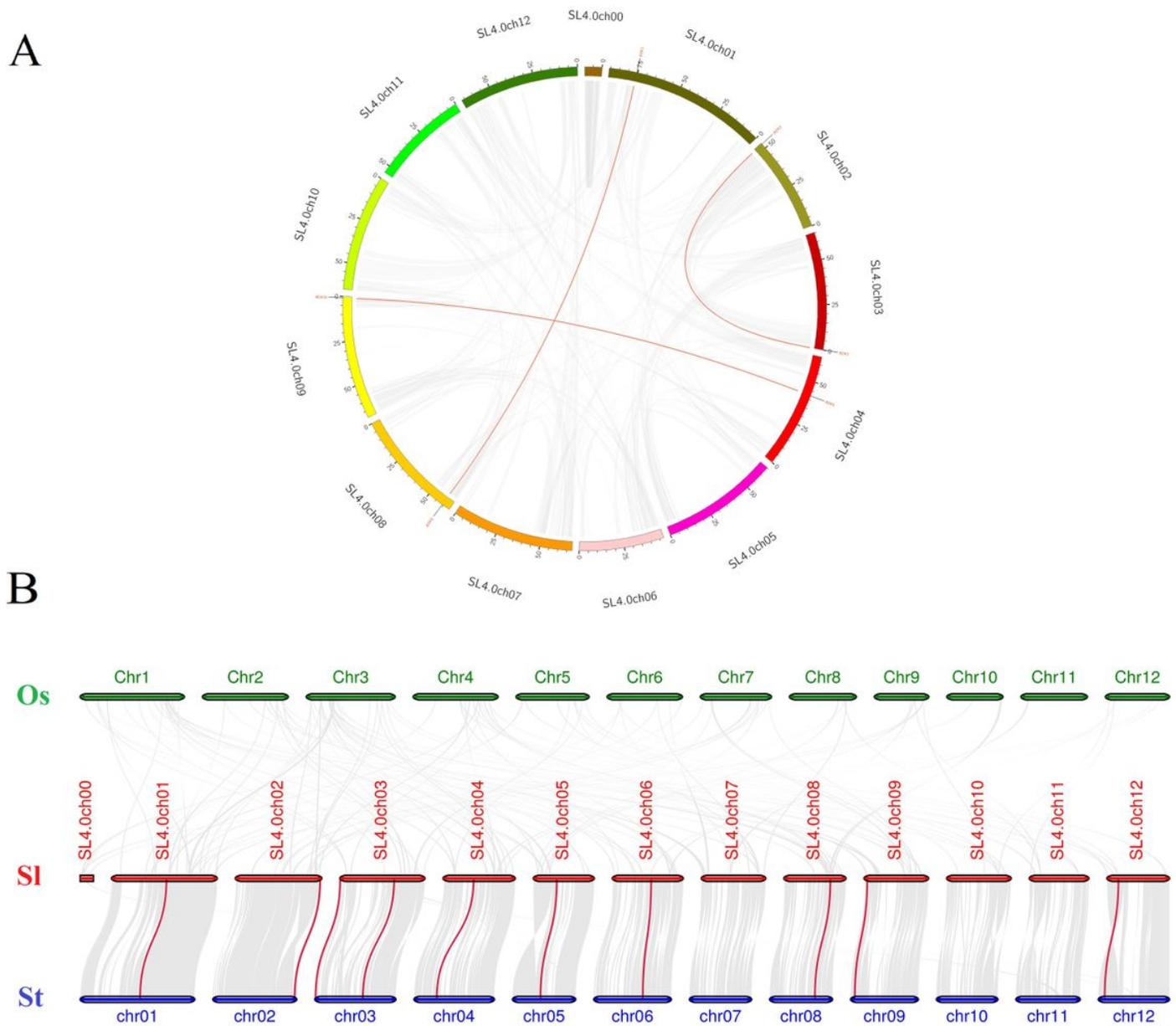


Figure 4

Collinearity analysis of ADK gene family. A. Synteny analysis of ADK family in tomato. The gray lines represent the collinearity result of tomato genome and red lines linking SIADK genes represent the duplication events occurred in tomato ADK gene family. Chromosomes were drawn in different colors and the approximate location of SIADK genes is shown by short black lines on the circle. B. Synteny analysis of ADK genes between tomato and other two representative plant species. The gray lines represented the collinear blocks within tomato and other plant genomes (potato, rice), while the red lines highlighted the syntenic ADK gene pairs. Here the abbreviation 'Os', 'Sl' and 'St' indicate *Oryza sativa*, *Solanum lycopersicum*, and *Solanum tuberosum*, respectively.

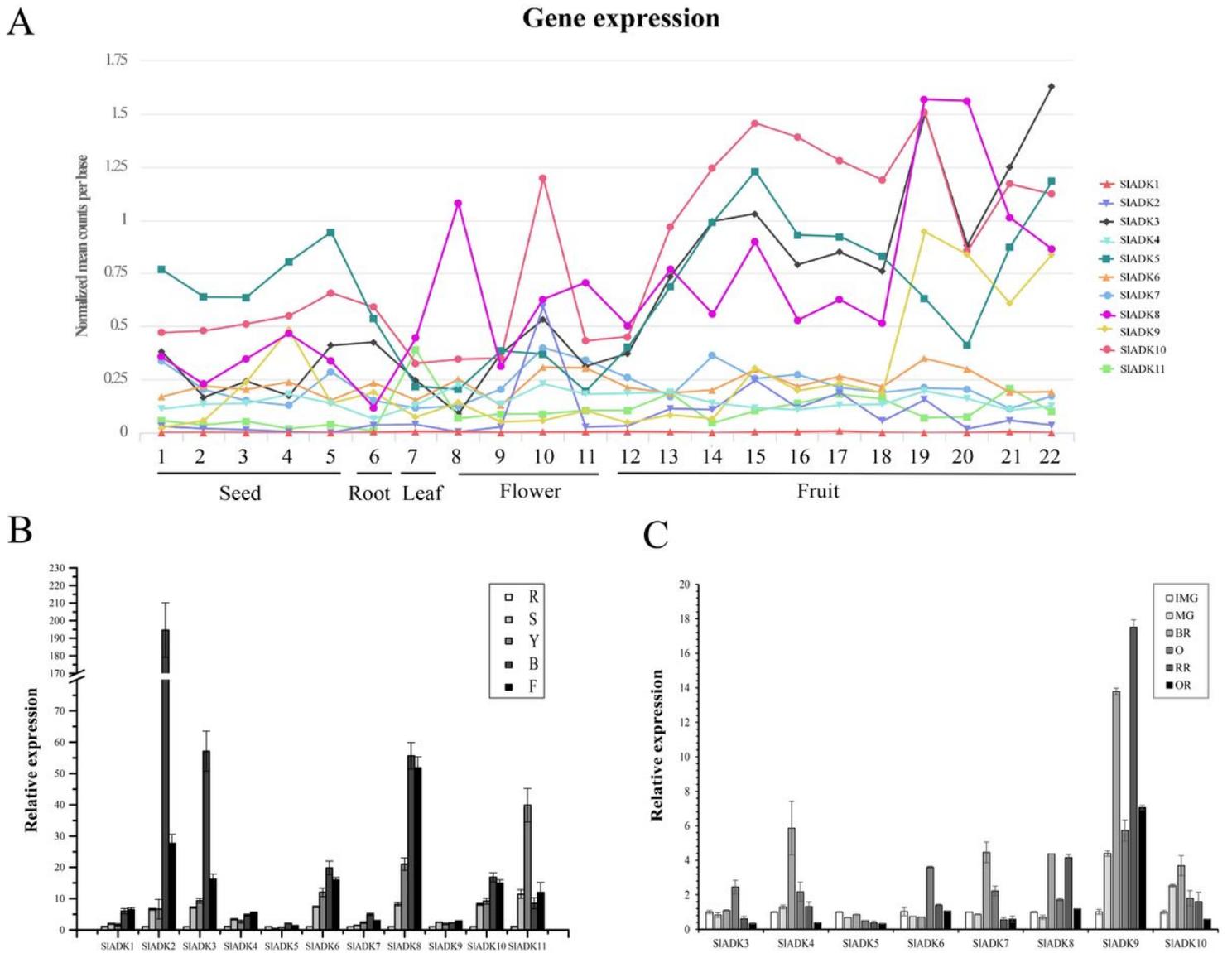


Figure 5

Expression patterns of the SIADK genes in different tissues. A. Expression patterns of the SIADK genes in various tissues obtained from the TomExpress platform. 1, whole seed of immature green fruit (10 day post anthesis, DPA); 2, whole seed of mature green fruit (35 DPA); 3, whole seed of breaker fruit (38 DPA); 4, whole seed of orange fruit (41 DPA); 5, whole seed of red fruit (44 DPA); 6, whole root; 7, whole leaf; 8, petal of flower; 9, whole flower in the stage of bud; 10, whole flower in the stage of bud in 3mm; 11, whole flower in the stage of anthesis; 12, whole fruit in the stage of 4 DPA; 13 and 14, flesh and peel of immature green fruit (10 DPA), respectively; 15 and 16, flesh and peel of mature green fruit (35 DPA), respectively; 17 and 18, flesh and peel of breaker fruit (38 DPA), respectively; 19 and 20, flesh and peel of orange fruit (41 DPA), respectively; 21 and 22, flesh and peel of red fruit (44 DPA), respectively. B. Expression of SIADK genes with real-time PCR detection in various tissues including roots (R), stems (S), fully expanded leaves (L), bud (B) and flowers (F). C. Expression of SIADK genes with real-time PCR detection at different stages during fruit development and ripening: immature green (IMG), mature green

(MG), breaker (Br), orange (O), red ripe (R) and over ripe (OR). Quantitative PCR data represent mean values for three independent biological replicates (n = 3).

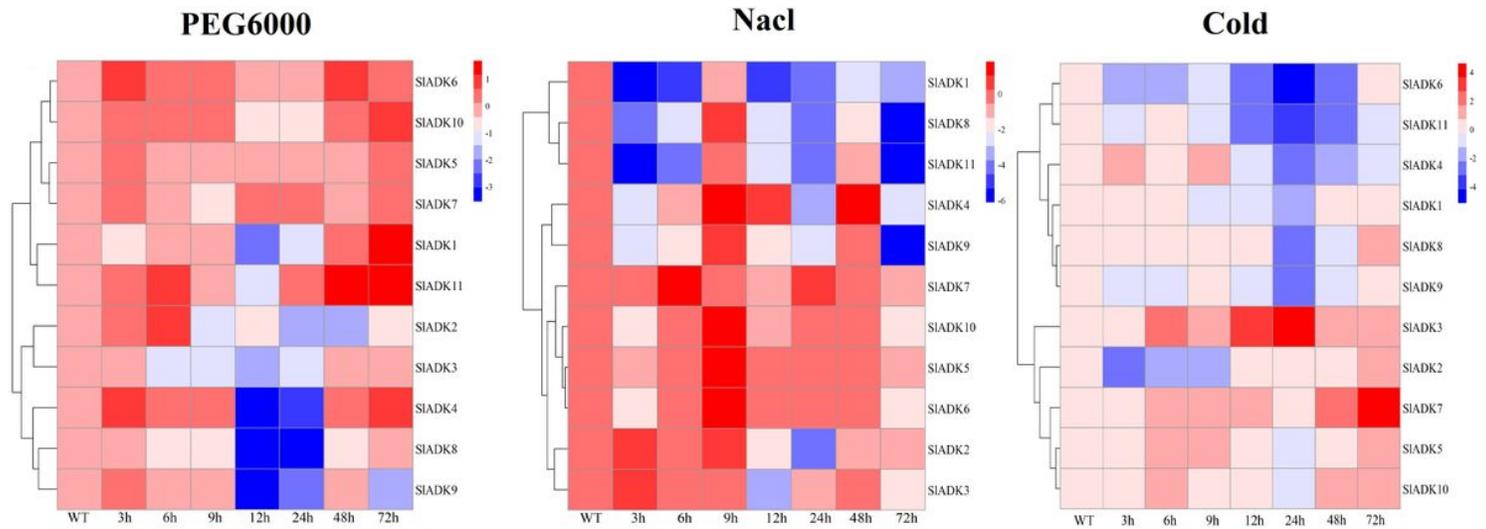


Figure 6

qRT-PCR expression analysis for 11 SIADKs in tomato leaves under diverse abiotic stresses (PEG6000, NaCl, Cold). The treated tomato leaves were harvested at the indicated time points. The color scale represents log₂ mean value of relative expression levels from three independent biological replicas (n = 3). Used primers specific for SIADKs were listed in Additional file Table S5.

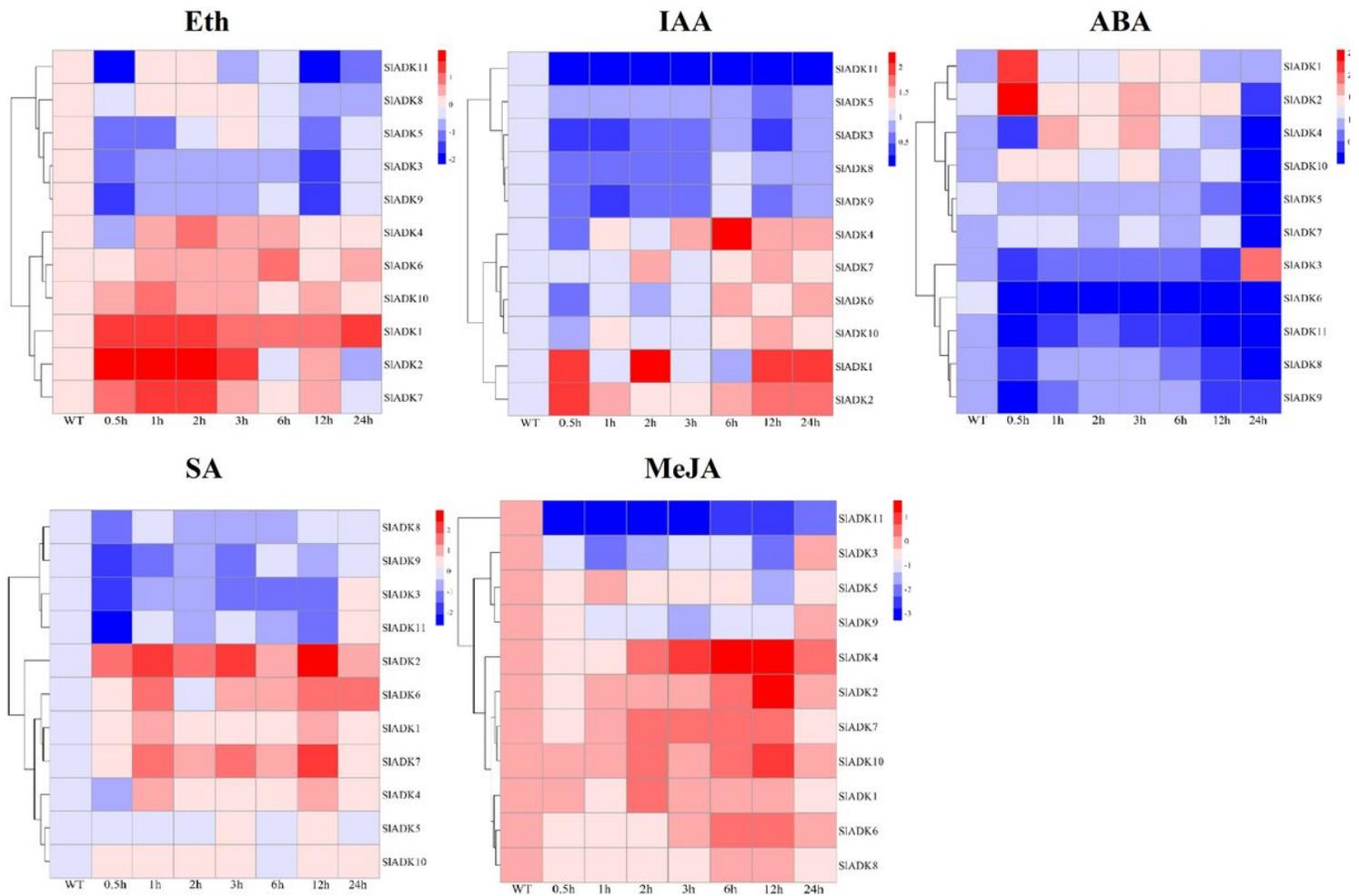


Figure 7

Expression profiles of SIADKs under various hormone treatments by qRT-PCR. The treated tomato leaves were harvested at the indicated time points. To ensure expression of the same gene in different treatments as well as expression of different genes in the same treatment can both be clearly displayed and compared, for the treatment of Eth, SA and MeJA, the color scale represents log₂ mean value of relative expression levels from three independent biological replicas (n = 3); For the treatment of IAA and ABA, the color scale represents mean value of relative expression levels from three independent biological replicas (n = 3). Primers specific for SIADKs were listed in Additional file TableS5.

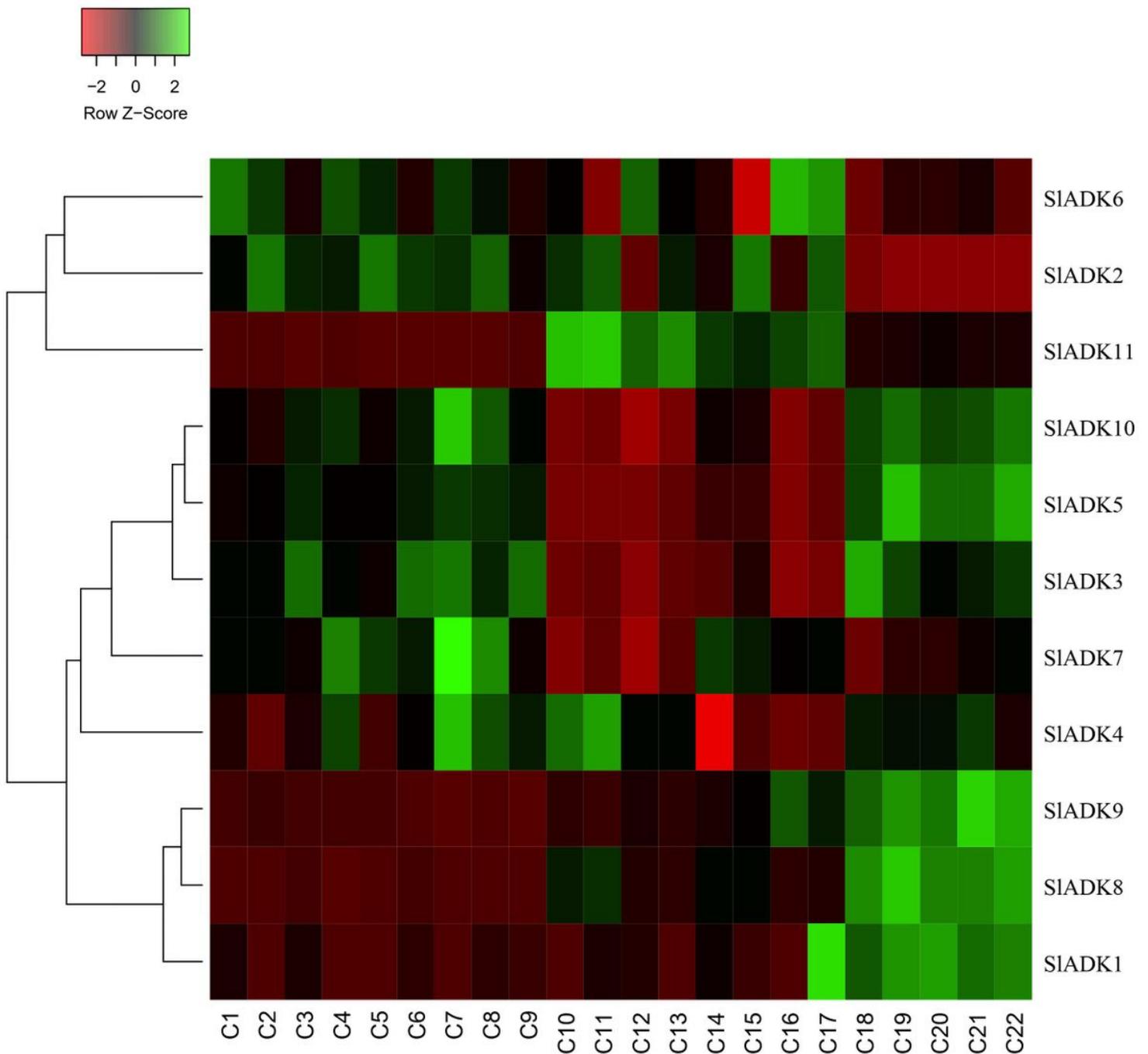


Figure 8

Expression patterns of SIADK genes under stress based on RNA-seq data. Heatmap of the expression pattern of tomato SIADK family genes in root, leaves and fruit with different and multiple hormones treatment. The distance used for the clustering is based on the classical Euclidean distance which allows clustering gene expression by expression levels. For a given row of the heatmap, green and red colors present high and low values of expression of the considered gene, respectively. Global condition information of different samples from C1 to C22 could be found in Fig. S2.

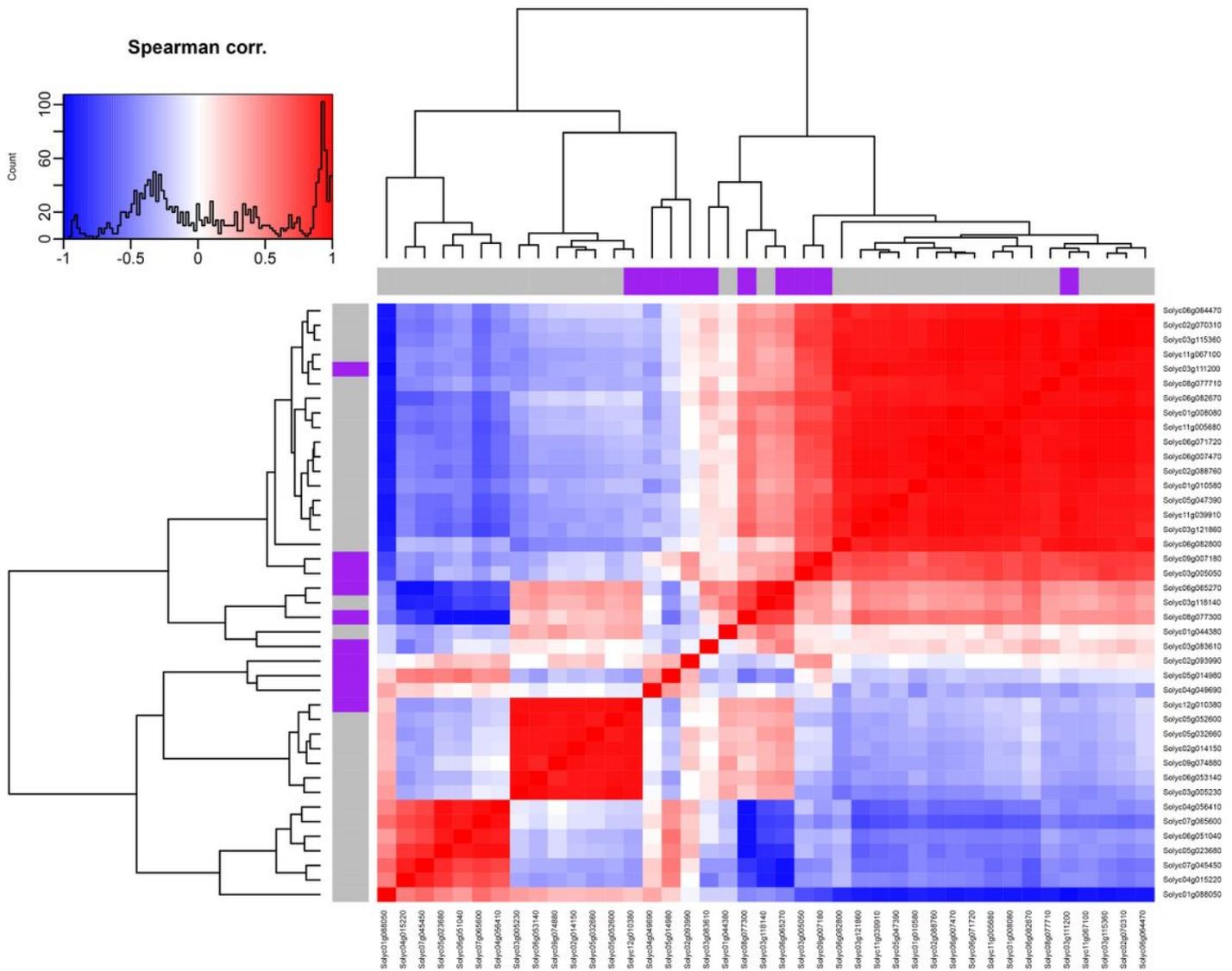


Figure 9

Correction heatmap of SIADK genes. Based upon the calculation of the correlation values, correlation gene pairs were visualized as a heatmap after a hierarchical clustering to highlight the positive and negative correlated groups. For a given row of the heatmap, red and blue colors present positive and negative correlation coefficient, respectively. The diagonal line from the lower left corner to the upper right corner of the heat map showed the same gene, and the default correlation coefficient is 1.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [TableS1CDSandgenessequenceofSIADKs.doc](#)
- [TableS2ADKAAsequencesofdifferentplantspecies.doc](#)
- [TableS3Featuresofn motifs of SIADK.docx](#)

- [TableS5PrimerssequencesofSIADKsusedforqPCR.doc](#)
- [TableS4DetailedinformationofCREinSIADKpromoters.xlsx](#)
- [TableS6Coexpressionandcorrelationnetworkanalysis.xlsx](#)
- [FigureS1.jpg](#)
- [FigureS2.jpg](#)
- [Table2.PredictedsubcellularlocationofSIADKs..xls](#)