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

Jiaxin Xiao

Anhui Normal University

Zhengguo Li (✉ zhengguoli@cqu.edu.cn)

Chongqing University

Research article

Keywords: Genome-wide, Tomato, ADK gene family, Stress

Posted Date: August 21st, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-57426/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Version of Record: A version of this preprint was published at International Journal of Molecular Sciences on July 19th, 2021.
See the published version at <https://doi.org/10.3390/ijms22147708>.

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 was divided into five 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. The 11 *SIADKs* were randomly distributed on nine of the 12 tomato chromosomes. A cis-element analysis inferred that several stress response elements were found on the promoters of *SIADKs*. 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. For example, almost all *SIADKs* contained two expression peaks at 9 and 48 h following salt treatment. 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.

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 genetic material in cells. Moreover, it is the energy transfer substance 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 Ca^{2+}/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 (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 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). 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. After germination of seeds in water, transfer them into soil 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. Well-grown tomato plants about one month were selected and transplanted from soil to hydroponic box for hydroponic culture, 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. After treatment, tissue of leaves was collected from three individual plants and mixed thoroughly, then frozen in liquid nitrogen immediately and stored at $-80\text{ }^{\circ}\text{C}$.

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 these two methods, combining gene description in SOL Genomics Network and National Center for Biotechnology Information (NCBI) database.

Characteristics, phylogenetic relationship and sequence analysis of ADK proteins

Seven adenylate kinase isoforms have been found with high sequence homology in Arabidopsis genome [14]. Then the full-length ADK proteins in Arabidopsis and identified in tomato 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 sequence 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.nbcr.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. 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, the software CELLO v.2.5: subcellular localization predictor (<http://cello.life.nctu.edu.tw/>) was used.

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

Visualizing the gene structure and annotated features help for determining their function and evolution 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].

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 [32, 33]. To assess the 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 later.

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 µM ethephon (Eth), 100 µM abscisic acid (ABA), 500 µM salicylic acid (SA) and 50 µM 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, culturing the plants without watering for continual 15 days. Well-watered plants were maintained as controls. At each treatment, materials from three separate plants were combined to form one sample, and all of experiments for treatment were performed in three independent times. All these samples were frozen in liquid nitrogen immediately and stored at -80 °C until RNA extraction.

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 S3) 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 by means $\pm$ standard deviation (SD).

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) and subcellular localization are listed in Table 1. 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*) (Table 1) and the corresponding range for MWs was 22872.10-74110.62 Da. 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*). The CELLO software tool for prediction of intracellular localization was used. Most *SIADK* proteins were presumably cytosolic (e.g. *SIADK1*, *4*, *8*, *10* and *11*); *SIADK2*, *5* and *7* were predicted to be located in mitochondria or cytosolic; and *SIADK6* was predicted as located in the nucleus or cytosolic. Interestingly, *SIADK3* was presumably in cytosolic, mitochondria or nucleus.

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

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

Phylogenetic analysis and multiple sequence alignment of *SIADK* genes

The phylogenetic relationship of tomato *SIADK* proteins, together with seven Arabidopsis *AtADKs* were examined by multiple sequence alignment of 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* gene family was divided into five groups (Fig. 1A). Genes *SIADK6* and *10* together with two Arabidopsis homologs (AT2g39270 and AT2g37250) were phylogenetically distinct and formed the predicted first branch of plant *ADKs*. The second branch contained one isoforms of Arabidopsis (AT3g01820) and two of tomato (*SIADK2* and *3*). The third branch contained two isoforms of Arabidopsis (AT5g50370 and AT5g63400) and two of tomato (*SIADK5* and *7*). The fourth branch contained two evolutionarily similar subbranches: *SIADK8* and AT5g47840; and *SIADK11* and AT5g35170. The more distantly related sequences were the previously characterized tomato *SIADK1*, *4* and *9*, which formed as branch V. 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. The conserved domain which belonged to motif 2 is marked.

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 some *SIADKs* (e.g. *SIADK1* and *9*, *SIADK2* and *3*, and *SIADK5* and *7*) shared common motif compositions, indicating potential functional similarities among these proteins. Motifs 1, 2 and 4 were shared by all

SIADKs, indicating fundamental functions of these motifs in ADK proteins. 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.

Gene structure and chromosomal location analysis of *SIADKs*

Gene organization plays a vital role in the evolution of multiple gene families [34]. The neighbor-joining phylogenetic tree constructed with MEGA7 is shown in Fig. 3A. 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. The average gene length was 1610–9218 bp. The largest genes were *SIADK6* and *11*, and the longest was *SIADK11*, which had the maximum number of exons; *SIADK2*, *3* and *10* were 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 S1). The 11 *SIADKs* were randomly distributed on nine of the 12 tomato chromosomes (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. Detailed results can be found in Table S2. Eight cis-regulatory elements with high frequency of occurrence on *SIADK* promoters (ABA-responsive element (ABRE), AT ~ TATA-box, Box, CAAT-box, G-box, MYB, MYC and TATA-box) were analyzed (Fig. 3C). Generally, TATA-box and CAAT-box which can promote transcription appeared most frequently on the *SIADK* promoters. The upstream regulatory sequence of promoters contained multiple elements that respond to hormones (such as Box and G-box) and stress signals (such as 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 -500 bp, cis-regulatory elements of *SIADK11* and *1* were less distributed and almost nonexistent, respectively.

Expression patterns prediction 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. 4A). 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*, *7* 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. 4B 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 (Fig. 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. 4C).

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. 5). Following 3 h of treatment with PEG6000 (an osmotic agent used to simulate drought stress), 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.

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 [35–37]. Here, we investigated the expression profiles of *SIADKs* in response to Eth, IAA, ABA, SA and MeJA treatments (Fig. 6). With Eth treatment, the expression of *SIADK4*, 5, 6, 8, 9 and 11 decreased 30 min following the initial treatment, while expression of *SIADK1*, 2, 7 and 10 increased at the first time point. 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 of most genes remained stable with time. Notably, 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 30 min 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. Gene *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 30 min. 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. Notably, *SIADK2* and 4 exhibited significant changes under multiple hormone treatments, suggesting that they may have unique roles in hormone regulation.

Discussion

ADK is ubiquitous in the kingdoms Animalia and Plantae. ADK 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. 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 [38]. The *SIADKs* and *AtADKs* were classified into five groups, with genes in the same group showing a closer evolutionary relationship. For example, *SIADK1*, 4 and 9 belonged to group V; *SIADK6* and 10 to group I; and *SIADK8* and 11 to group IV. Much closer evolutionary relationships existed in the same subbranch, such as *SIADK2* with 3, *SIADK5* with 7, and *SIADK1* with 9 (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 1). This is consistent with previous reports that ADK activity in plants is mainly distributed in the chloroplast matrix and mitochondrial membrane space [39, 40, 41].

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 [42]. For the evolution of multiple gene families, the model of gene organization is very important [34]. Gene structure analysis revealed 4–19 exons in each *SIADK* (Fig. 3A). All above mentioned genes in the same subbranch (Fig. 1A), 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*.

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. 4A, 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. 4A, 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. 4B).

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% [43]. 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. 5). 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, 44]. 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 (U214214) 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. Genes *SIADK3* and 7 responded strongly to cold treatment, indicating that they may play a role in cold stress (Fig. 5).

In addition to the basic transcription elements TATA-box and CAAT-box distributed in multiple sites, most of the *ADK* family members also contain some specific cis-regulatory elements, such as ABRE, MYB, G-box and MYC (Table S2, 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 S2, 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 regulate the induced expression of ABA by these 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).

Previous evidence indicated that different hormones play important roles in cell responses and stress signal transduction [35–37]. For example, IAA is involved in almost all aspects of plant growth and development, from embryogenesis to senescence, from root tip to shoot tip [53]. Eth is a key regulator during fleshy fruit ripening [54]. ABA is a crucial phytohormone induced by biotic or abiotic stress, and plays important roles in plant tolerance to abiotic stresses [55]. MeJA play an important role in alleviating biotic (pathogens and insects) and abiotic stresses in plants [56]. Our study showed that the transcripts of these *SIADKs* were responsive to most hormone treatments (Fig. 6). 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 [57]. Also, both Eth and SA play important roles in biotic stresses [58]. Notably, *SIADK2* and 4 exhibited significant changes under these hormone treatments, suggesting that they have unique roles in hormone responsiveness (Fig. 6). So, understanding the response of *SIADKs* to hormones can lay a foundation for further elucidating their functions in plant growth and stress response.

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 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. 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:override

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

All data generated or analysed 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

Funding

This research was supported by the 17th Huo Yingdong Education Fund Project (171022), Anhui Provincial Natural Science Foundation (2008085QC129), and the Provincial Natural Science Research Program of Higher Education of Anhui province (KJ2019A0484).

Authors' contributions

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

Acknowledgements

The authors thank to lab members for assistance

References

1. Dzheia PP, Kal'venas AA, Toleikis AI, Prashkiavichius AK. The role of adenylate kinase in the regulation of the rate and effectiveness of energy transfer from mitochondria to hexokinase in vitro. *Biokhimiia*. 1986;51(6):974–9.
2. Atkinson DE. The energy charge of the adenylate pool as a regulatory parameter. Interaction with feedback modifiers. *Biochemistry*. 1968;7:4030–4.
3. Ching TM, Crane JM. Adenylate energy pool and energy charge in maturing rape seeds. *Plant Physiol*. 1974;54:748–51.
4. Raymond P, Pradet A. Stabilization of adenine nucleotide ratios at various values by an oxygen limitation of respiration in germinating lettuce (*Lactuca sativa*) seeds. *Biochem J*. 1980;190(1):39–44.
5. Chang HY, Fu CY. Adenylate Kinase. *Encyclopedia of Food Microbiology*. 2014;1:16–24.
6. Noda LH. Adenylate kinase. In: Boyer PD, editor. *The Enzymes (Group Transfer Part A)*. New York: Vol VII. Academic Press; 1973. pp. 279–305.
7. Pradet A, Raymond P. Adenine nucleotide ratios and adenylate energy charge in energy metabolism. *Ann Rev Plant Physiol*. 1983;34:199–224.
8. Maragakis P, Karplus M. Large amplitude conformational change in proteins explored with a plastic network model: adenylate kinase. *J Mol Biol*. 2005;352(4):807–22.
9. Arora K, Brooks CL. Large-scale allosteric conformational transitions of adenylate kinase appear to involve a population-shift mechanism. *Proc Natl Acad Sci U S A*. 2007;104(47):18496–501.
10. Kawai M, Kidou S, Kato A, Uchimiya H. Biology M. Molecular characterization of cDNA encoding for adenylate kinase of rice (*Oryza sativa* L.). *Plant J*. 1992;2(6):845–54.
11. Chen Z, Fu H, Liu D, Chang PF, Narasimhan M, Ferl R, et al. A NaCl-regulated plant gene encoding a brain protein homolog that activates ADP ribosyltransferase and inhibits protein kinase C. *Plant J*. 1994;6(5):729–40.
12. Regierer B, Fernie AR, Springer F, Perez-Melis A, Leisse A, Koehl K, et al. Starch content and yield increase as a result of altering adenylate pools in transgenic plants. *Nat Biotechnol*. 2002;20(12):1256–60.
13. Carrari F, Coll-Garcia D, Schauer N, Lytovchenko A, Palacios-Rojas N, Balbo I, et al. Deficiency of a plastidial adenylate kinase in *Arabidopsis* results in elevated photosynthetic amino acid biosynthesis and enhanced growth. *Plant Physiol*.

2005;137(1):70–82.

14. Lange PR, Geserick C, Tischendorf G, Zrenner R. Functions of chloroplastic adenylate kinases in Arabidopsis. *Plant Physiol.* 2008;146(2):492–504.
15. Zhang YZ, Launay H, Liu F, Lebrun R, Gontero B. Interaction between adenylate kinase 3 and glyceraldehyde-3-phosphate dehydrogenase from *Chlamydomonas reinhardtii*. *FEBS J.* 2018;285(13):2495–503.
16. Peterson TA, Nlieman RH, Clark RA. Nucleotide metabolism in salt-stressed *Zea mays* L. Root tips: I. Adenine and uridine nucleotides. *Plant Physiol.* 1987;85(4):984–9.
17. Zhou S, Wei S, Boone B, Levy S. Microarray analysis of genes affected by salt stress in tomato. *Afr J Environ Sci Technol.* 2007;1(2):014–26.
18. Gong P, Zhang J, Li H, Yang C, Zhang C, Zhang X, et al. Transcriptional profiles of drought-responsive genes in modulating transcription signal transduction, and biochemical pathways in tomato. *J Exp Bot.* 2010;61(13):3563–75.
19. Raveneau MP, Benamar A, Macherel D. Water content, adenylate kinase, and mitochondria drive adenylate balance in dehydrating and imbibing seeds. *J Exp Bot.* 2017;68(13):3501–12.
20. Giovannoni J. Molecular biology of fruit maturation and ripening. *Annu Rev Plant Physiol Plant Mol Biol.* 2001;52:725–49.
21. Tomato Genome Consortium. The tomato genome sequence provides insights into fleshy fruit. *evolutionNature.* 2012;485:635–41.
22. Finn RD, Coghill P, Eberhardt RY, Eddy SR, Mistry J, Mitchell AL, Potter SC, Punta M, Qureshi M, Sangrador-Vegas A, Salazar GA, Tate J, Bateman A. The Pfam protein families database: towards a more sustainable future. *Nucleic Acid Res.* 2016;44:D279–85.
23. Johnson LS, Eddy SR, Portugaly E. Hidden Markov model speed heuristic and iterative HMM search procedure. *BMC Bioinform.* 2010;11:431.
24. Letunic ISMART. Available online: <http://smart.embl-heidelberg.de/> (accessed on 16 March 2017).
25. Kumar S, Stecher G, Tamura K. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol.* 2016;33:1870–4.
26. Hall BG. Building phylogenetic trees from molecular data with MEGA. *Mol Biol Evol.* 2013;30(5):1229–35.
27. Bailey TL, Boden M, Buske FA, Frith M, Grant CE, Clementi L, Ren J, Li WW, Noble WS. MEME SUITE: tools for motif discovery and searching. *Nucleic Acids Res.* 2009;37:W202–8.
28. Artimo P, Jonnalagedda M, Arnold K, Baratin D, Csardi G, de Castro E, Duvaud S, Flegel V, Fortier A, Gasteiger E, Grosdidier A, Hernandez C, Ioannidis V, Kuznetsov D, Liechti R, Moretti S, Mostaguir K, Redaschi N, Rossier G, Xenarios I, Stockinger H. ExpASY: SIB bioinformatics resource portal. *Nucleic Acids Res.* 2012;40(Web Server issue):W597–603.
29. Hu B, Jin J, Guo AY, Zhang H, Luo J, Gao G. GSDS 2.0: an upgraded gene feature visualization server. *Bioinformatics.* 2015;31(8):1296–7.
30. Voorrips RE. MapChart: software for the graphical presentation of linkage maps and QTLs. *J Hered.* 2002;93(1):77–8.
31. Lescot M, Déhais P, Thijs G, Marchal K, Moreau Y, Yves VDP, Pierree R, Stephane R. Plantcare, a database of plant cis-acting regulatory elements and a portal to tools for in silico analysis of promoter sequences. *Nucleic Acids Res.* 2002;30(1):325–7.
32. Liu M, Gomes BL, Mila I, Purgatto E, Peres LE, Frasse P, Maza E, Zouine M, Roustan JP, Bouzayen M, Pirrello J. Comprehensive profiling of ethylene response factor expression identifies ripening-associated ERF genes and their link to key regulators of fruit ripening in Tomato. *Plant Physiol.* 2016;170(3):1732–44.
33. Zouine M, Maza E, Djari A, Lauvernier M, Frasse P, Smouni A, Pirrello J, Bouzayen M. Tomexpress, a unified tomato RNA-Seq platform for visualization of expression data, clustering and correlation networks. *Plant J.* 2017;92(4):727–35.
34. Xu G, Guo C, Shan H, Kong H. Divergence of duplicate genes in exon-intron structure. *Proc Natl Acad Sci U S A.* 2012;109(4):1187–92.

35. Lorenzo O, Solano R. Molecular players regulating the jasmonate signalling network. *Curr Opin Plant Biol.* 2005;8:532–40.
36. Mauch-Mani B, Mauch F. The role of abscisic acid in plant-pathogen interactions. *Curr Opin Plant Biol.* 2005;8:409–14.
37. Hirayama T, Shinozaki K. Research on plant abiotic stress responses in the post-genome era: past, present and future. *Plant J.* 2010;61:1041–52.
38. Zhao P, Wang D, Wang R, Kong N, Zhang C, Yang C, et al. Genome-wide analysis of the potato Hsp20 gene family: identification, genomic organization and expression profiles in response to heat stress. *BMC Genom.* 2018;19(1):61.
39. Birkenhead K, Walker D, Foyer C. The intracellular distribution of adenylate kinase in the leaves of spinach, wheat and barley. *Planta.* 1982;156(2):171–5.
40. Hampp R, Goller M, Ziegler H. Adenylate levels, energy charge, and phosphorylation potential during dark-light and light-dark transition in chloroplasts, mitochondria, and cytosol of mesophyll protoplasts from *Avena sativa* L. *Plant Physiol.* 1982;69(2):448–55.
41. Stitt M, Lilley RM, Heldt HW. Adenine nucleotide levels in the cytosol, chloroplasts, and mitochondria of wheat leaf protoplasts. *Plant Physiol.* 1982;70(4):971–7.
42. Wang X, Xue S, Chen S, Ma C, Xu S. Evolutionary origin, gradual accumulation and functional divergence of heat shock factor gene family with plant evolution. *Front Plant Sci.* 2018;9:71.
43. Bray EA, Bailey-Serres J, Weretilnyk E. Responses to abiotic stress. In: Buchanan B, Gruissem W, Jones R, eds. *Biochemistry and molecular biology of plants.* The American Society of Plant Physiologists. 2000; 1158–1203.
44. Atkin OK, Macherel D. The crucial role of plant mitochondria in orchestrating drought tolerance. *Ann Bot.* 2009;103(4):581–97.
45. Shinozaki K, Yamaguchi-Shinozaki K. Molecular responses to drought and cold stress. *Curr Opin Biotechnol.* 1996;7(2):161–7.
46. Bray EA. Plant responses to water deficit. *Trends Plant Sci.* 1997;2(97):48–54.
47. Zhu JK. Salt and drought stress signal transduction in plants. *Annu Rev Plant Biol.* 2002;53:247–73.
48. Shinozaki K, Yamaguchi-Shinozaki K. Gene networks involved in drought stress response and tolerance. *J Exp Bot.* 2007;58(2):221–7.
49. Shen Q, Ho TH. Functional dissection of an abscisic acid (ABA)-inducible gene reveals two independent ABA-responsive complexes each containing a G-box and a novel cis-acting element. *Plant Cell.* 1995;7(3):295–307.
50. Yamaguchi-Shinozaki K, Urao T, Shinozaki K. Regulation of genes that are induced by drought stress in *Arabidopsis thaliana*. *J Plant Res.* 1995;108(1):127–36.
51. Agarwal M, Hao Y, Kapoor A, Dong CH, Fujii H, Zheng X, et al. A R2R3 type MYB transcription factor is involved in the cold regulation of CBF genes and in acquired freezing tolerance. *J Biol chem.* 2006;281(49):37636–45.
52. Onishi M, Tachi H, Kojima T, Shiraiwa M, Takahara H. Molecular cloning and characterization of a novel salt-inducible gene encoding an acidic isoform of PR-5 protein in soybean(*Glycine max* [L.] Merr.). *Plant Physiol Biochem.* 2006;44(10):574–80.
53. Woodward AW, Bartel B. Auxin: regulation, action, and interaction. *Ann Bot.* 2005;95(5):707–35.
54. Kumar R, Khurana A, Sharma AK. Role of plant hormones and their interplay in development and ripening of fleshy fruits. *J Exp Bot.* 2014;65(16):4561–75.
55. Lee SC, Luan S. ABA signal transduction at the crossroad of biotic and abiotic stress responses. *Plant Cell Environ.* 2012;35(1):53–60.
56. Yu X, Zhang W, Zhang Y, Zhang X, Lang D, Zhang X. The roles of methyl jasmonate to stress in plants. *Funct Plant Biol.* 2019;46(3):197–212.
57. Tanaka Y, Sano T, Tamaoki M, Nakajima N, Kondo N, Hasezawa S, et al. Ethylene inhibits abscisic acid-induced stomatal closure in *Arabidopsis*. *Plant Physiol.* 2005;138(4):2337–43.

58. Fujita M, Fujita Y, Noutoshi Y, Takahashi F, Narusaka Y, Yamaguchi-Shinozaki K, Shinozaki K. Crosstalk between abiotic and biotic stress responses: a current view from the points of convergence in the stress signaling networks. Curr Opin Plant Biol. 2006;9(4):436–42.

Figures

Figure 1

Phylogenetic analysis and multiple sequence alignment of adenylate kinase (ADK). A. Phylogenetic analysis of tomato ADK gene family members, together with 7 Arabidopsis ADKs, all of which were 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 genes. A. Details of ten selected motifs. Ten conserved motifs of SIADKs 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. Distributions of conserved motifs in SIADK genes. Ten putative motifs were indicated in different colored boxes.

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 upstream/downstream 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 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

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, seed of immature green fruit (10 day post anthesis, DPA); 2, seed of mature green fruit (35 DPA); 3, seed of breaker fruit (38 DPA); 5, seed of orange fruit (41 DPA); 6, seed of red fruit (44 DPA); 6, root; 7, leaf; 8, flower; 9, bud; 10, bud in 3mm; 11, flowers during anthesis; 12, 4 DPA fruit; 13 and 14, immature green fruit (10 DPA, two different cultivated plants); 15 and 16, mature green fruit (35 DPA); 17 and 18, breaker fruit (38 DPA); 19 and 20, orange fruit (41 DPA); 21 and 22, red fruit (44 DPA). 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 5

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. Data indicated relative expression levels (means $\pm$ SE) from three independent biological replica ($n = 3$). Used primers specific for SIADKs were listed in Additional file TableS3.

Figure 6

Expression profiles of SIADKs under various hormone treatments by qRT-PCR. The treated tomato leaves were harvested at the indicated time points. Data indicate relative expression levels (means $\pm$ SE) from three independent biological replica ($n = 3$). Used primers specific for SIADKs were listed in Additional file TableS3.

Supplementary Files

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

- [TableS3PrimerssequencesofSIADKsusedforqPCR.doc](#)
- [TableS2DetailedinformationofCREinSIADKpromoters.xlsx](#)
- [TableS1CDSandgenesequenceofSIADKs.doc](#)