Molecular and Phylogenetic Analyses of the Mediator Subunit Genes in Solanum lycopersicum

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The Mediator complex is a multi-subunit protein assembly that serves as a central scaffold to help regulate DNA-binding transcription factors (TFs) and RNA polymerase II (Pol II) activity controlled gene expression programmed in response to developmental or environmental factors. However, litter information about Mediator complex subunit (MED) genes in tomato is available, although it is an essential model plant. In this study, we retrieved 46 candidate SIMED genes from the genome of tomato, and a comprehensive analysis was conducted, including their phylogenetic relationship, chromosomal locations, gene structure, cis-regulatory elements prediction, as well as gene expression. The expression profiling of 46 SIMED genes was analyzed using publicly available RNA-seq data. Furthermore, we selected some SIMED genes to evaluate their expression patterns in various tissues and under different abiotic stress treatments by quantitative reverse transcription PCR experiments. This is the first detailed report to elucidate the molecular and phylogenetic features of the MED genes in tomato, and it provides valuable clues for further functional analysis in order to clarify the role of the SIMED genes in diverse plant growth, development and abiotic stress response.

Keywords: Mediator complex subunits, Solanum lycopersicum, genomic characterization, expression analysis, plant stress response

INTRODUCTION

In eukaryotes, transcription is primarily controlled by RNA polymerase II (Pol II) (Sikorski and Buratowski, 2009). Transcription by PolII is an intricate process that requires a large number of transcription factors (TFs), including DNA-binding TFs, general transcription factors (GTFs), as well as transcription coactivators (Davidson and Bolouri, 2002; Levine and Tjian, 2003). Mediator, a multi-subunit protein complex, is the central coactivator that acts as a bridge between DNA-binding TFs and the basal Pol II machinery assembled at the core promoter region (Kim et al., 1994; Kornberg, 2005; Borggreve and Yue, 2011). The Mediator complex was originally identified and purified in Saccharomyces cerevisiae and was found to be required for the activator-dependent stimulation of transcriptional activation (Kelleher et al., 1990; Flanagan et al., 1991). Subsequent investigation showed that Mediator is evolutionarily conserved from yeast to higher organisms (Boube et al., 2002). The number of Mediator complex subunits can vary from 25 to 35 depending upon the species, and the yeast Mediator complex comprises 25 subunits. More than 30 different Mediator complex subunits have been described in different organisms, but only approximately 20 have been found in all eukaryotes (Conaway and Conaway, 2011b). Based on biochemical and
structural studies, Mediator is organized into four separate domains (head, middle, tail, and a detachable kinase module). The primary function of the tail domain is its involvement in the interaction with the DNA-bound transcriptional regulators (TFs), and the head and the middle domains interact with the Pol II-TEFIIF complex and C-terminal domain (CTD) of Pol II, respectively (Ansari and Morse, 2013). The evidence from genetic experiments suggests that the whole Mediator complex acts as a central component of the transcription machinery. Additionally, individual Mediator subunits also have significant gene-specific and even tissue-specific functions (Conaway and Conaway, 2011a).

In yeast and animals, Mediator subunits have been shown to have critical functions in cell and organismal viability (Tudor et al., 1999), embryonic viability (Ito et al., 2000; Gillmor et al., 2010; Risley et al., 2010), organ development (Rau et al., 2006), as well as human immunity and diseases (Spaeth et al., 2011). In plants, the research on the Mediator complex is relatively backward. In 2007, the Mediator complex of Arabidopsis thaliana has been successfully purified (Bäckström et al., 2007). Subsequently, some plant Mediator subunits (MED) have been functionally characterized and several plant MED genes have been demonstrated to have important regulatory roles in plant development, flowering, the regulation of hormone signaling pathways, and biotic and abiotic stress tolerance (Kidd et al., 2011). The Arabidopsis Mediator subunits, namely, AtMED12 and AtMED13, mediate the timing of embryo patterning (Gillmor et al., 2010); AtMED14 controls cell proliferation and shoot meristem development (Autran et al., 2002); AtMED25 regulates multiple pathways, such as hormone signaling pathways, flowering time, and abiotic and biotic stress responses (Yu and Lin, 2005; Kidd et al., 2009; Dorcafernall et al., 2011); AtMed17, AtMed18, and AtMed20 promote the transcription of miRNA and are responsible for the morphological development (Yun et al., 2011); AtMED8 functions in the production of root hairs (Sundaravelpandian et al., 2013); and AtMED16 affects iron homeostasis and is required for plant defence signalling crosstalk (Wathugala et al., 2012; Yan et al., 2014; Wang et al., 2015). Moreover, the functions of the Mediator subunits have also been established in other model plants. For instance, OsMED4 has been proposed to be involved in regulating rice tiller growth (Li et al., 2008); OsMED15 has been hypothesized to control seed development and seed size (Thakur et al., 2013); and NtMed8 is a key regulator of tobacco vegetative development and floral organ size (Wang et al., 2011). Additionally, we found that SlMED18 can regulate the development of leaves and stems in tomato (Wang et al., 2018).

Tomato (Solanum lycopersicum) is one of the most widely consumed vegetables and considered an essential model plant for studying plant development and fruit ripening (Consortium, 2012). Thus, a comprehensive analysis of all SlMED genes is really necessary. In this study, to advance our understanding of the evolution and functions of the SlMED genes, we investigated whole SlMED genes by using bioinformatic analyses and identified 46 MED genes in tomato. Further analysis of these genes' structure, chromosome distribution, exon-intron organization, as well as the comprehensive protein sequence analysis and phylogenetic comparison were carried out. In order to reveal the expression pattern of SlMED genes in different organs and under various abiotic stress conditions, we obtained a pre-expression pattern of all of the SlMED genes and analyzed their regulatory promoter elements. Furthermore, the expression pattern of several SlMED genes was determined by quantitative real-time PCR analyses in different stages of plant development, especially in fruit ripening. We also characterized their expression under various abiotic stress conditions to confirm the reliability of our predicted results. These results provide details of the tomato Mediators, which will be useful for future cloning and the functional characterization of the MED genes.

METHODS

Identification of SlMED Genes

In this study, a total of 46 MED proteins were identified by BLASTP search (e-value was set at 1e-5), and protein sequences of Arabidopsis and rice MED were used as queries to retrieve among the SGN (Solanaceae Genomics Network) (https://solgenomics.net/) and NCBI (National Center for Biotechnology Information) (http://www.ncbi.nlm.nih.gov/) database. The A. thaliana MED proteins were searched from the FAIR (The Arabidopsis Information Resource) database (http://www.arabidopsis.org/) and the protein information of rice (Oryza sativa) was downloaded from RGAP (Rice Genome Annotation Project) (http://rice.plantbio.msu.edu/index.shtml), based on previous reports (Saloni et al., 2011; Dolan and Chapple, 2016). Putative MED proteins have been further identified by Hidden Markov Model (HMM) methods using each individual Med subunit domain. All candidate protein sequences were examined using PROSITE (http://expasy.org/tools/protein.html) and SMART (http://smart.embl-heidelberg.de/) database for reliability. Full-length protein, DNA, and CDS (coding DNA sequence) sequence of SlMED were downloaded from the SGN. The molecular weight and isoelectric points of tomato MED proteins were detected by the ExPASy proteomics server (http://www.expasy.org/).

Protein Alignment and Phylogenetic Analysis

The full-length amino acid sequences of Arabidopsis, rice, and tomato were aligned using ClustalX 1.81 (http://www.clustal.org/) (Saitou, 1987). Unrooted phylogenetic trees were generated by MEGA5.02 program, in which the evolutionary history can be inferred by the neighbor-joining method. The best DNA/protein models we found was “JTT+G” and the Bootstrap analysis was performed using 1,000 replicates (Tamura et al., 2011; Bast, 2013).

Gene Structure Analysis

The GSDS (Gene Structure Display Server program) software (http://gsds.cbi.pku.edu.cn) was used to reveal the exon/intron structures for individual tomato MED genes by comparison of CDS and corresponding genomic DNA sequences from SGN and NCBI (Hu et al., 2014).
Chromosomal Localization
In order to determine the chromosomal locations of tomato MED genes, we obtained the starting and ending positions of all candidate genes on each tomato chromosome from the SGN and NCBI database. The physical map was drawn using the Tomato-EXPEN 2000 (https://solgenomics.net/cview/map.pl) and the resulting chromosome position of each candidate gene was indicated by gene name.

Digital Gene Expression Analysis
For the digital expression profiles of SIMED genes, the standardized expression levels of candidate genes, based on the RNA-seq data, were downloaded from wild species LA1589 (Solanum pinnellifolium) date in digital expression (RNA-seq) dataset of TFGD (the Tomato Functional Genomics) (http://ted.bti.cornell.edu/cgi-bin/TFGD/digital/home.cgi) database.

Moreover, the data of Tomato Lab (http://tomatolab.cshl.edu/~lippmanlab2/allexp_query.html) and Tomato eFP Browser (http://bar.utoronto.ca/efp_tomato/cgi-bin/efpWeb.cgi) were used to further validate the expression pattern of SIMED. Gene expression levels in the different tissues were calculated according to RPKM (reads per kilobase million) values of RNA-seq data. The RPKM values were displayed in Supplementary Table S2. A heat map was generated using MEV 4.9.0 (multiple experiment viewer) (http://www.tm4.org/mev.html) to visualize the expression profiles of the MED genes in different tomato organs (Howe et al., 2010; Howe et al., 2011).

Promoter cis-Acting Regulatory Element Analysis
The promoter sequences (3,000 bp upstream 5’UTR region) were searched from NCBI database using CDS of SIMED genes as the queries. Then the PlantCare database (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) was used to find the cis-acting regulatory elements of different hormones and stress-responsive in SIMED genes.

Plant Material and Growth Conditions
In this experiment, AC++ (S. lycopersicum, “Ailsa Craig” AC++) (Peralta et al., 2005), near to isogenic lines containing rin (ripening inhibitor) (Knapp et al., 1989) and Nr (Never-ripe) (Thompson et al., 1999) were cultivated in a greenhouse with long day conditions (16-h light/25°C, 8-h dark/18°C). Tissues from roots, stems, leaves, sepals, flowers, and fruits of different stages were gathered from adult tomato plants (70-day-old tomato). The flowers were marked at anthesis and the ripening periods of fruits were recorded according to days post-anthesis (DPA). Tomato fruit ripening stages were divided from green fruits to ripe fruits, including IMG (immature green), MG (mature green), B (breaker), B+4 (4 days after breaker), and B+7 (7 days after breaker). After tissue collection, we frozen all plant samples in liquid nitrogen quickly and then stored at −80°C until RNA isolation.

Plant Hormone and Stress Treatments
For the plant hormone and stress treatment, AC++ seeds were germinated with seedlings cultivated in the greenhouse (Peralta et al., 2005). Then, we selected the uniform potted 35-day-old tomato seedlings for diverse treatments. For the dehydration stress, the seedlings were carefully pulled out from the soil and cleaned cautiously using water until the soil was removed. Then they were put on dry filter paper at 25 ± 1°C and the leaves were harvested at different periods (Pan et al., 2012). For hormone treatment, the plant seedlings were sprayed, respectively, with H2O, 100 μM ABA, 50 μM MeJA, 100 μM ACC, and 50 μM GA3 solution (Zhu et al., 2014), enclosed in plastic instantly after spraying and treated after 0, 1, 2, 4, 8, 12, 24 h. Then the leaves of seedlings were gathered for study. All tissues were frozen in liquid nitrogen immediately, then stored at −80°C for the experiment.

Extraction of Total Ribonucleic Acid and Analysis of Quantitative Real-Time Polymerase Chain Reaction
Total RNA was extracted from all mentioned plant tissues in this article using TRizol reagent (Invitrogen, Shanghai, China). After DNase digestion (Promega), cDNA was synthesized with oligo(dT)20 as primer by RNA that reverse-transcribed using Moloney murine leukemia virus reverse transcriptase (Promega). The final tube of 10 μl quantitative reverse transcription PCR (qRT-PCR) reaction system included 1 μl cDNA as template, 0.25 μl 10 mM each primer, 5 μl 2× Go-Taq qPCR Master Mix (Promega, Beijing, China), and 3.5 μl distilled water. qRT-PCR was accomplished using the CFX96™ Real-Time System (Bio-Rad, USA) under the following conditions: 95°C 2 min, 40 cycles of 95°C 15 s, followed by 60°C 35 s and a melting curve was created and analyzed. The 2-ΔΔCt method was used to compare the expression levels of candidate genes, based on the RNA-seq data. The RPKM values were displayed in Supplementary Table S2. A heat map was generated using MEV 4.9.0 (multiple experiment viewer) (http://www.tm4.org/mev.html) to visualize the expression profiles of the MED genes in different tomato organs (Howe et al., 2010; Howe et al., 2011).

Statistical Analyses
The mean values of data were calculated from three replicates and presented as mean ± standard deviation. The t-test (**P < 0.01 and *P < 0.05) was used to analyze the significant differences. The Origin 8.0 software was used to perform the data analysis.

RESULTS
Identification of the Mediator Complex Subunit Genes in Tomato
To identify the MED genes in tomato, keyword and BLAST searches were performed against the SGN and NCBI databases.
A total of 46 MED proteins were identified. For convenience, the tomato MED genes were named based on the NCBI database (Table 1). Further analysis indicated that the coding region length was in the range of 359 (SIMED11) to 6,789 bp (SIMED12), which encoded polypeptides that varied from 99 aa with a molecular weight of 16.4 kDa to 2,262 aa with a molecular weight of 250.6 kDa. SIMED36 had the highest isoelectric point (10.12), while SIMED21 had the lowest isoelectric point (4.46). Detailed information for the MED genes in tomato, including their names, accession numbers, molecular details, their homologous protein in Arabidopsis as well as Mediator modules, are listed in Table 1.

### Phylogenetic Relationship of Mediator Complex Subunit Genes

To analyze the phylogenetic relationships between tomato, Arabidopsis and rice MED proteins, multiple sequence alignments among 46 predicted tomato MED proteins, 49 Arabidopsis MED proteins, and 40 rice MED proteins were performed using MUSCLE (Figure 1). The tree was constructed using the neighbor-joining method with the Jukes-Cantor correction and the maximum-likelihood method with 1,000 bootstrap replicates. The results showed that the MED proteins can be divided into three groups: the kinase group, the catalytic core group, and the regulatory subunit group. The kinase group includes the SlMED16, SlMED20a, SlMED28, and SlMED19a genes, which are homologous to the Arabidopsis AtMED16 and AtMED28 genes. The catalytic core group includes the SlMED11, SlMED12, SlMED13, SlMED14, and SlMED15 genes, which are homologous to the Arabidopsis AtMED12, AtMED13, AtMED14, and AtMED15 genes. The regulatory subunit group includes the SlMED21, SlMED22, SlMED23, and SlMED24 genes, which are homologous to the Arabidopsis AtMED21, AtMED22, AtMED23, and AtMED24 genes.

**Table 1** Overview of Mediator complex subunit genes identified in tomato. List of predicted genes and related information include sequenced ID, molecular details, their homologous protein in Arabidopsis thaliana, as well as Mediator modules.

| Gene name | Accession number | Coding region length (bp) | Protein | Arabidopsis homologous | Accession number | E-value | Mediator Module |
|-----------|------------------|---------------------------|---------|------------------------|------------------|---------|-----------------|
| SIMED2/32 | Solyc08g078204.2 | 519                       | 172     | 18,496.62              | AMED2/32         | 1e−64   | Tail            |
| SIMED3/27a| Solyc07g072030.2 | 1,236                      | 411     | 45,129.97              | AMED3/27         | 2e−167  | Tail            |
| SIMED3/27b | Solyc05g047740.1 | 768                       | 256     | 28,807.74              | AMED2/32         | 1e−48   | Tail            |
| SIMED4   | Solyc02g087180.2 | 1,275                      | 425     | 46,404.32              | AMED4            | 3e−180  | Middle          |
| SIMED5/33a| Solyc01g080200.3 | 3,957                      | 1,318   | 143,986.90             | AMED5a/33a       | 0.0     | Tail            |
| SIMED5/33b| Solyc06g009680.4 | 4,011                      | 1,336   | 145,367.17             | AMED5a/33a       | 0.0     | Tail            |
| SIMED5/33c| Solyc06g064780.2 | 3,966                      | 1,321   | 142,416.94             | AMED5a/33a       | 0.0     | Tail            |

**Note:** The table includes predicted genes and related information such as sequenced ID, molecular details, their homologous protein in Arabidopsis thaliana, as well as Mediator modules.
performed, and an N-J phylogenetic tree was constructed (Figure 1). The results illustrated that most of the MED proteins from tomato, *Arabidopsis*, and rice clustered with comparably high linkage on the phylogenetic tree such as SIMED6/AtMED6/OsMED6, SIMED17/AtMED17/SIMED17, SIMED18/AtMED18/OsMED18, and SIMED36/AtMED36/SIMED36. While the MED proteins among tomato clustered with little bootstrap support, and the MED proteins were not conserved across the same Mediator module. These results suggested close homology relationship and evolutionary conservation among MED proteins in plants.

**Mediator Complex Subunit Genes**

**Structure and Chromosomal Location**

The exon-intron structure of all 46 SIMED genes was analyzed according to their genome sequences and corresponding coding sequences using the online tool GSDS (Figure 2). The result showed that the structure of these genes varied among members. The number of introns varied from 0 to 28. SIMED3/27a do not contain an intron in their genomic sequences, whereas SIMED35b has 28 introns. Consequently, the number of exons ranged from 1 to 29. Seven members have a simple structure with three exons. While most SIMED genes possess more than three exons.

To characterize the chromosomal distribution of the SIMED genes, the physical locations of 46 related genes on the tomato chromosomes were analyzed according to the genome sequencing information from the SGN database. As illustrated in Figure 3, SIMED genes were represented on almost all chromosomes. Furthermore, there were five SIMED genes distributed on chromosomes 1, 3, 4, 6, and 8; four SIMED genes located on chromosomes 2, 5, 10, and 12; two SIMED genes mapped to chromosomes 9 and 11; and only one SIMED gene assigned to chromosome 7 (Figure 3).

**Ribonucleic Acid Sequencing Expression Analyses of Mediator Complex Subunit Genes in Tomato**

Since high-throughput sequencing and gene transcription analyses have been conducted on various tomato tissues at different developmental stages, publicly available RNA-seq data are considered to be a useful resource for analyses gene expression profiles. Tomato transcript expression (RNA-seq)
data in eight different tomato tissues, including roots (RT), young leaves (YL), mature leaves (ML), young flower buds (YFB), fully open flowers (FL), 10 days post-anthesis fruits (10 DPA), 20 days post-anthesis fruits (20 DPA), and mature fruits (MF), were used to investigate the expression profiles of \textit{SlMED} genes. A hierarchical clustering heat map was constructed that displayed the expression patterns of 46 \textit{SlMED} genes (Figure 4). Most of the \textit{SlMED} genes exhibited distinct expression profiles across the eight tissues examined, indicating that different \textit{SlMED} genes might function in diverse ways to regulate tomato growth and development. It is interesting to note that the expression levels of \textit{SlMED2/32}, \textit{SlMED15}, \textit{SlMED23}, \textit{SlMED35b}, and \textit{SlCycC} were relatively higher in all eight tissues. However, three genes, \textit{SlMED20b}, \textit{SlMED20c}, \textit{SlMED20d}, \textit{SlMED20e}, and \textit{SlMED34}, showed low levels of transcription in every organ tested. Specific \textit{MED} genes, \textit{SlMED3/27b} and \textit{SlMED37}, were abundantly expressed in MF but had extremely lower transcript levels in other tissues. This finding suggested that these two genes might function in regulating tomato fruit growth and development.

**Promoter Structure Analysis of Tomato Mediator Complex Subunit Genes**

The analysis of promoter structure is thought to be one of the most important ways to predict the promoter regions and expression profiles of a gene, as well as to reveal hidden transcriptional networks. In this study, we identified 3 kb promoter sequences of putative \textit{SIMED} genes. Bioinformatic analyses of these sequences with the PlantCare database illuminated typical regulatory elements of plant gene promoters, containing elements that respond to phytohormone and abiotic stresses. The number of elements specifically responsive to plant hormones and abiotic stresses was identified in \textit{SIMED} gene promoter regions as shown in Figure 5. Moreover, the names, position and their functional descriptions of each regulatory
elements were listed in Supplementary Table S3. This analysis indicated that the promoter of each SIMED gene contained more than two important putative regulatory elements, implying that the expression of SIMED genes might be regulated by different phytohormones and abiotic stresses. For abiotic stresses, the regulatory elements for heat and dehydration stress were abundantly found in SIMED genes, while fewer elements for low temperature were identified. Additionally, several cis-acting motifs responsible for six kinds of phytohormones MeJA (methyl jasmonate), SA (salicylic acid), ABA (abscisic acid), GA (gibberellin), IAA (auxin), ET (ethylene), were also enriched. Most low-temperature responsive elements were discovered in the promoters of SIMED21. Seven regulatory elements required for dehydration were identified in the promoters of SIMED9 and SIMED21. The promoters of SIMED17, SIMED22, and SIMED23 contained a large number of regulatory elements necessary for MeJA, indicated they may play a role in MeJA stress response. SIMED17 and SIMED37 were observed to have more ABA-responsive elements in their promoters than the other SIMED genes. Moreover, six GA response elements were found in the promoter region of SIMED9, four were discovered in SIMED20b, three were found in SIMED21.

Expression Profiles of Selected Tomato Mediator Complex Subunit Genes in Different Tissues by Quantitative Reverse Transcription Polymerase Chain Reaction

According to the results of Figure 4, most tomato MED genes have abundant expression in diverse organs, except SIMED3/27b, SIMED5/33a, and SIMED37 showed special expression trend during fruit development. To validate the gene expression profiles in diverse organs with the RNA-seq database, nine SIMED genes, including SIMED3/27b, SIMED5/33a, and SIMED37, were selected to confirm their expression in roots (RT), YL, ML, senescent leaves (SL), sepal of flower in anthesis (SE), flowers (FL), immature green fruits (IMG), mature green fruits (MG), breaker fruits (B), 4 days after breaker fruits (B+4), and 7 days after breaker fruits (B+7), using quantitative RT-PCR (Figures 6A–I). As was expected, most genes showed highly similar expression profiles between the RNA-seq data and qRT-PCR data. SIMED11 was more highly expressed in 20 DPA according to the RNA-seq data, whereas the qRT-PCR data indicated that it was more highly expressed in B+7 (Figure 6C). Otherwise, the RNA-Seq expression patterns showed that SIMED17 expression level was higher in RT and YFB, which was inconsistent with the qRT-PCR data that displayed the highest expression level in B+7 (Figure 6D). These conflicting results between our RNA-seq data and qRT-PCR data might be due to the difference of plants growth conditions and experimental conditions. From these results, it can be speculated that some SIMED genes with different expression levels across multiple tomato tissues may have unique functions in plant specific development.

Notably, SIMED3/27b and SIMED37 showed organ-preferential expression, which was expressed specifically and strongly in MF (Figures 6A, I). However, the expression of SIMED5/33a gradually decreased during the fruit growth and ripening stages (Figure 6H). Thus, further analysis of the transcriptional accumulation of SIMED3/27b, SIMED5/33a, and SIMED37 from the IMG to B + 7 stages in normal Nr mutant and rin mutant fruits was conducted to investigate whether or not these three genes are related to the ripening-deficient mutants (Figures 6J–L). In wild type fruits and the Nr mutant, the expression of SIMED3/27b subsequently increased during fruit ripening and showed the highest level at the B+7 stage,
while in the rin mutant the expression of \textit{SIMED3/27b} indicated a decreased trend during fruit ripening (Figure 6J). A dissimilar expression trend was shown among the wild type, \textit{Nr} and rin fruits of \textit{SIMED5/33a}, indicating that \textit{SIMED5/33a} expression may be impacted by \textit{RIN} and \textit{Nr} (Figure 6K). Additionally, \textit{SIMED37} expression was found at a high level in B, B+4, and B+7 fruits in wild type, whereas its expression in \textit{Nr} and rin was at nearly the same level during five stages of fruit development and ripening (Figure 6L). These data suggested that \textit{SIMED37} may play a significant role in fruit ripening.

### Expression Pattern of Tomato Mediator Complex Subunit Genes Under Various Phytohormones and Abiotic Stresses

From the promoter structure analysis of \textit{SIMED} genes, we found these genes may have potential roles in response to various abiotic stresses. To gain more insight into the response and regulation of \textit{SIMED} genes under abiotic stresses and hormone treatment, we selected some \textit{SIMED} genes with a large number of regulatory elements in their promoter sequences and observed the expression profiles of these genes under dehydration,
MeJA, ABA, GA, IAA, and ACC treatments using qRT-PCR (Figure 7). The expression levels of most of the genes examined changed greatly following exposure to these treatments. As shown in Figure 7A, SIMED9, SIMED21, and SIMED22 had no significant changes in response to dehydration stress, while the expression level of SIMED26b was dramatically upregulated by dehydration stress (Figure 7A). Under the GA treatment, SIED3/27b, SIMED21, SIMED22, and SIMED25a were expressed at relatively low levels (Figures 7B, E, F, H). For ACC treatment, the level of SIED3/27b first declined, but after 4-h treatment, its expression returned to its original level (Figure 7B). In contrast, SIMED37 was markedly upregulated at 2 and 4 h (Figure 7I). Interestingly, we found that SIMED17, SIMED21, and SIMED23 were upregulated more than two-fold in response to MeJA after 8-h treatment (Figures 7C, E, G). The SIMED18 gene expression decreased remarkably following ABA treatment (Figure 7D). However, there was no significant expression level change in SIMED37 following ABA stress treatment (Figure 7I).

DISCUSSION

Characterization of Mediator Complex Subunit Genes in Tomato

The Mediator complex serves an essential function in gene regulation, acting as a bridge between DNA-bound TFs and Pol II initiation machinery. Recently, reports revealed not only that the Mediator complex could regulate TFs expression but also that the individual Mediator subunit may have specific roles in plant development and abiotic stress responses (Hentges, 2011). In plants, molecular and phylogenetic analyses of MED genes have only been performed in Arabidopsis and rice (Bäckström et al., 2007; Mathur and Tyagi, 2011). Nevertheless, little is known about MED genes in tomato.

The comprehensive identification of the evolution, structure, and expression of SIMED genes provides new insight into their potential role. In this study, we first identified 46 SIMED genes through a genome-wide analysis, indicating that the numbers
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of tomato MED gene members were contracted compared to Arabidopsis (49) (Table 1). In tomato, the number of MED7, MED10, MED15, MED22, MED26, MED37, and CycC homolog proteins were less than Arabidopsis, whereas the tomato genome has more MED19, MED20, and MED3/27 homolog genes. The loss of MED genes during evolution suggests that the function of some MED genes may be replaced by their homolog genes. And it is possible that more MED19, MED20, and MED3/27 homolog genes are needed in tomato genome. In the phylogenetic tree, most tomato MED genes closely related to the Arabidopsis and rice homologues, indicating that the MED genes of tomato, Arabidopsis, and rice may have shared a close evolutionary relationship (Figure 1). This result was similar to previous reports that MED genes are conserved across the plant kingdom (Mathur and Tyagi, 2011). Some individual Mediator subunits have several homologue genes such as SlMED19a, SlMED19b, and SlMED19c sharing evolutionary origins which may play similar physiological functions. Additionally, the SlMED genes have various numbers of exons, implying diversity present in genes structure among SlMED genes. We found that several SlMED genes, such as SlMED23 and SlMED35b, have long introns (Figure 2). These findings indicate structural differences and diversity in the SlMED genes.

In the plant kingdom, some reports have proven that MED genes function in multiple stages of plant development (Kidd et al., 2011; Lai et al., 2014a; Samanta and Thakur, 2015). In Mediator complex, the requirement for individual MED subunits varies. Some subunits are essential elements of Mediator architecture which are broadly required for Mediator function, while others only function in specific organs or pathways. Analysis of the tissue specific expression of SlMED genes is useful for evaluating their underlying biological functions in different organs. As a

FIGURE 6 | Quantitative PCR (qPCR) analysis of selected SlMED genes in different tissues. (A–I) Relative expression of selected SlMED genes in different developmental stages and tissues by qPCR analysis. The results were expressed using the root (Rt) as a reference for each gene (relative messenger RNA level 1). (J-L) Expression patterns of selected SlMED genes in wild type and ripening mutant fruits. The tissues included roots (RT), young leaves (YL), mature leaves (ML), senescent leaves (SL), sepal of flower in anthesis (SE), flowers (FL), immature green fruits (MG), mature green fruits (MG), breaker fruits (B), 4 days after breaker fruits (B+4), and 7 days after breaker fruits (B+7). Each value represents the mean ± SD of three replicates.
Figure 7 | Expression patterns of the SlMED genes in response to abiotic stress treatments. (A) Effect of dehydration on the expression of SlMED9, SlMED21, SlMED22, and SlMED26b genes in leaves by quantitative PCR analysis. (B-I) Expression profiles of the SlMED genes after various phytohormones treatment. Each value represents the experiment among three independent biological repetitions. Bars indicate the SEM of three experimental repetition.
result, tomato transcript expression (RNA-seq) data were used to reveal the expression profiles of SIMED genes. We found that among the 46 predicted genes, most genes were highly expressed in all of the tissues tested, whereas SIED3/27b and SIMED37 were specifically expressed in fruit ripening stages (Figure 4). In tomato, we found five MED20 paralogs (SIMED20a, SIMED20b, SIMED20c, SIMED20d, SIMED20e) which is more than in Arabidopsis. The transcript expression (RNA-seq) data showed that only SIMED20a had higher relative expression levels than other four paralogs. It is possible that SIMED20a plays a major role in tomato. The SIMED20b, SIMED20c, SIMED20d, and SIMED20e may have a function at some special growth stages or under particular environmental conditions. To further validate the expression profiles in different tissues gained from RNA-seq database, the expression of nine SIMED genes was detected by qRT-PCR in roots (RT), YL, ML, senescent leaves (SL), sepal of flower in anthesis (SE), flowers (FL), IMG, mature green fruits (MG), breaker fruits (B), 4 days after breaker fruits (B + 4), and 7 days after breaker fruits (B+7) (Figure 6). Furthermore, the statistical correlation (R value) between the relative expression values of the qRT-PCR results and the log2 RPKM values from the RNA-seq analysis were calculated and they were compared well, except for two genes (SIMED11 and SIMED17) (Supplementary Table S2). The conflicting results between qRT-PCR date and RNA-seq data might be due to differences in plant materials, growth conditions, and experimental conditions. Both RNA-seq data and qRT-PCR result showed that SIMED18 was abundantly expressed in all the tissues we examined. In our previous report, we found that the repression of SIMED18 caused multiple plant developmental defects, and it was involved in the regulation of numerous plant growth and development processes in tomato, which was consistent with the expression profiles (Wang et al., 2018). These results may indicate the reliability of RNA-Seq expression patterns.

Potential Functions of SIMED Genes During Fruit Development

Tomato ripening is a complex and highly coordinated developmental process associated with various physiological and biochemical modifications such as changes in colour, flavour, sugar, organic acids, as well as nutrient composition (Klee and Giovannoni, 2011). This process requires the activity of a series of TFs. The central coactivator complex, the Mediator complex, which acts as a bridge to transfer the message between TFs and the basal Pol II machinery assembled at the core promoter region, may perform essential roles in the regulation of fruit ripening. It was noteworthy that the expression levels of SIED3/27b and SIMED37 were very high in fruit after B stage, while SIMED5/33a exhibited gradually decreased expression during the fruit growth and ripening stages (Figures 6A, H, I). To date, a quantity of ripening-deficient mutants, such as the never-ripe (Nr) and ripening inhibitor (rin) mutants have been identified and studied extensively in tomato. The Nr mutant is insensitive to ET and shows an incomplete and delayed ripening phenotype (Rick, 1956; Lanahan et al., 1994; Hackett et al., 2000), and the rin mutant shows negative effects on all measured ripening phenomena, including carotenoid biosynthesis, increased respiration and ET production, flavor compound synthesis, and fruit softening (Tigchelaar et al., 1978; Kitagawa et al., 2005). To determine whether these three genes were related to tomato fruit development and ripening, we further analyzed the expression of SIED3/27b, SIMED5/33a, and SIMED37 from the IMG to B + 7 stage in normal and ripening-deficient mutant (Nr and rin) fruits and discovered that the expression level of these three genes changed in different ripening-deficient mutants (Figures 6J–L). Thus, we speculate that SIED3/27b, SIMED5/33a, and SIMED37 may be associated with the process of fruit development and ripening in tomato.

Phytohormones and Abiotic Stress Responsive Mediator Complex Subunit Genes in Tomato

Plants perceive and integrate stress environmental signals, such as temperature, and dehydration, as well as various phytohormones, by different regulatory pathways. Several Arabidopsis MEDI genes have been shown to play key roles in the activation of stress signaling pathways. For example, AtMED16, AtMED14, and AtMED2 were demonstrated to regulate COLD ON-REGULATED (COR) genes and they were insensitive to cold stress (Hemsley et al., 2014). AtMED19a was reported to be a key regulator in ABA-mediated transcriptional regulation (Li et al., 2018). In addition, AtMED15 is known to act as a critical factor in the SA response (Canet et al., 2012).

The regulatory elements that are present in the promoter sequence, can bind a number of TFs and control gene regulation and expression. Observation of the promoter structure can support information on gene regulatory networks. According to promoter profiling analysis, we selected several SIMED genes with a large number of specific regulatory elements in the promoter sequences and investigated the expression profiles of these genes under various stress treatments using qRT-PCR. Dehydration is one of the most severe abiotic stress factors and is harmful to crop productivity. In this study, promoter profiling showed that two MBS (MYB binding site involved in drought-inducibility) elements, required for dehydration, were found in the promoters of SIMED26b. The MBS element is a general component in drought stress response genes (Abe et al., 1997). The response of plants to drought stress is likely to be dependent on the presence of such elements in specific gene promoters, so we speculated that SIMED26b might be a dehydration stress related gene in tomato. Additionally, qRT-PCR indicated that the expression level of SIMED26b was dramatically upregulated by dehydration stress (Figure 7A). This results also indicated that SIMED26b may be involved in the regulation of plant dehydration tolerance, potentially representing a new discovery of MED genes involved in dehydration stress. In addition, plant hormones are also well known to function in the regulation of plant growth and development. In view of the expression level of SIED3/27b after GA3 and ACC treatments together with the analyses of the regulatory elements, we propose that SIED3/27b may function as a regulator of GA3 and ACC signaling (Figure 7B). AtMED17, AtMED21, and AtMED23 have been reported to play important
roles in plant growth (Kidd et al., 2011). In tomato, these genes were upregulated in response to MeJA treatment, suggesting that they play potential roles in the resistance to MeJA stress (Figures 7C, E, G). Additionally, SlMED21 and SlMED22 showed reduced accumulation of mRNA under GA3 treatment, suggesting that negative control mechanisms might be present (Figures 7E, F). The expression level of SlMED18 was decreased remarkably by ABA treatment, which is consistent with the report in which the Arabidopsis med18 mutant showed ABA insensitivity (Lai et al., 2014a). In Arabidopsis, it was found that AtMED25 played a decisive role in JA signaling, whereas it had a negative effect on ABA signaling, which was also required for response to dehydration stress (Rong et al., 2012). Nevertheless, SlMED25a was induced by GA3 and contains four GA response elements in its promoter, implying that SlMED25a might have potential regulatory roles in GA stress responses (Figure 7H). SlMED37, a plant-specific Mediator subunit, was affected by ABA and ACC stresses in tomato (Figure 7I), suggesting their potential role under phytohormone stress. In particular, SlMED37 transcript level was remarkably increased by ACC and had the highest peak at 4 h. ACC is the immediate precursor of ET, and ET has been studied with respect to its critical roles in the ripening of fruit, the abscission of leaves, and abiotic stress adaptation. These results indicated that SlMED37 might act as an essential factor during plant development and in response to abiotic stresses. In the future, we intend to focus on identifying the function of these cascade SlMED genes. For example, we will verify whether SlMED26b is related to the dehydration tolerance by constructing a SlMED26b overexpression vector and generating transgenic overexpression tomato plants to perform a dehydration stress treatment. In addition, performing SlMED26b gene mutagenesis with the CRISPR/Cas9 system transformation method may also prove to be a helpful strategy. Thus, our promoter structure analysis and expression profiles under various abiotic stressed provide a foundation to study the role to SlMED genes in resistance of abiotic stress.

CONCLUSION

In conclusion, our study comprehensively performed a genome-wide analysis of tomato MED genes and provided systematic information about them. A total 46 of SlMED genes were identified, and their phylogenetic relationships with Arabidopsis, genomic organization, gene structure, cis-regulatory elements prediction, expression patterns among different tissues, and differential expression in response to phytohormones and abiotic stresses were characterized. In particular, SlMED37, SlMED33a, and SlMED37 were found to might have a role in fruit development and ripening. Furthermore, we revealed the putative functions of SlMED37, SlMED9, SlMED17, SlMED18, SlMED21, SlMED22, SlMED23, SlMED25a, SlMED26b, and SlMED37 in phytohormones and abiotic stress responses. These genes can be regarded as important candidates for further functional characterization. Taken together, our results will be helpful for obtaining a systematic understanding of SlMED genes and provide a useful reference for further functional studies of these genes.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

GC, ZH and QX designed and managed the research work and improved the manuscript. YuW and YiW performed the bioinformatics analysis. YuW, HL and CL performed the experiments. YuW wrote the manuscript. All authors reviewed the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2019.01222/full#supplementary-material

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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