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RESEARCH ARTICLE

Tomato COI gene family identification and expression under abiotic and phytohormone stress

MUHAMMAD WASEEM[1,2*], MEHTAB MUHAMMAD ASLAM[3,4] and IFFAT SHAHEEN[5]

[1]*College of Horticulture, South China Agricultural University, Guangzhou, People's Republic of China*
[2]*School of Life Sciences, Chongqing University, Huxi Campus, Daxuecheng, Shapingba 401331, People's Republic of China*
[3]*College of Agriculture, Yangzhou University, Yangzhou, Jiangsu, People's Republic of China*
[4]*College of Life Science, Fujian Agricultural and Forestry University, Fuzhou 350008, Fujian, People's Republic of China*
[5]*Faculty of Agriculture Science and Technology, Bahauddin Zakariya University, Multan, Pakistan*
*For correspondence. E-mail: Muhammad Waseem, mwasimbotanist@cqu.edu.cn, m.waseem.botanist@gmail.com.

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Abstract. The CORONATINE INSENSITIVE (COI) plays pivotal roles in plant growth and development, including pollen fertility, defence against pests, trichome formation, and seed germination. In this study, we performed bioinformatics characterization of COI proteins in tomato and analysed their expression profile analysis under abiotic stress. A total of nine members of the COI gene family were isolated and phylogenetically clustered into five distinct clades with *Arabidopsis*, rice, maize, and other related plant species. Subcellular localization showed selected COI proteins predominantly localized in the nucleus. The reverse transcription quantitative real-time polymerase chain reaction analysis revealed distinct spatial expression patterns SlCOIs among different tissues mainly found in the root and fruits of different developmental stages. In addition, we examined different hormone and abiotic stresses related to *cis*-regulatory sequences in upstream regions of these genes. Further, we examined differential changes in SlCOIs transcripts accumulation in response to different hormones (ABA, IAA, GA, SA and MeJA), salinity, drought, and cold. It was found that *SlCOI1*, *SlCOI2*, *SlCOI3*, *SlCOI4*, *SlCOI5* and *SlCOI7* was peaked under ABA, GA, SA and MeJA while, *SlCOI1*, *SlCOI3*, *SlCOI6* and *SlCOI8* were upregulated under salt, drought, and cold. These results provide invaluable insights into functional and protein functional features. Our research also provides a foundation for further functional characterization of COI genes in tomato.

Keywords. phytohormone; expression analysis; phylogeny; regulatory elements; salinity; tissue.

Introduction

Phytohormones influence distinct biological and developmental processes in plant ranging from seed germination to fruit ripening, and final seed dispersal (Goossens *et al.* 2016). Plant hormones such as jasmonate (JA), including jasmonic acid, methyl jasmonate (MeJA), and oxylipin derivatives control various developmental processes, including root formation (Wasternack and Hause 2013), cell cycle (Pauwels *et al.* 2008), resistance to pathogen invasion (Farmer *et al.* 2003), mechanical injuries (Devoto *et al.* 2005), leaf senescence (Weidhase *et al.* 1987), seed fertility and development (Xie *et al.* 1998; Qiang *et al.* 2014), and sex determination (Acosta *et al.* 2009). JAs are synthesised from linolenic acid, a precursor in JA biosynthesis. The beta-

Muhammad Waseem and Iffat Shaheen contributed equally to this work.

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oxidation of linolenic acid occurres in six steps, each catalysed by various enzymes such as allene oxide synthase (AOS), 12-oxo-phytodienoate reductase 3 (OPR3), and lipoxygenase (LOX) (Creelman and Mullet 1997; Wasternack and Hause 2013). JA signalling is mediated by a F-box component E3 ubiquitin- ligase SKP1-Cullin-F-box complex (SCF^{COI1}), CORONATINE INSENSITIVE 1 (COI1) (Yan *et al.* 2009), which ultimately lead to degradation of JAZ protein by 26S proteasome (Devoto *et al.* 2002; Xu *et al.* 2002; Chico *et al.* 2008).

JA responses have been well characterised through mutants analysis in Arabidopsis. For instance, Arabidopsis triple mutants (*fad3-2*, *fad7-2*, *coi1*) (Feys *et al.* 1994; McConn and Browse 1996) show reduced sensitivity to JA. The Arabidopsis JA-insensitive mutant *coi1* show pleiotropic phenotypes, including leaf yellowing (Castillo and Leon 2008; Reinbothe *et al.* 2009), susceptibility to pathogen invasion (Sanders *et al.* 2000). Moreover, Arabidopsis *COI1* involved apical dominance (Kim *et al.* 2013), root growth inhibition (Adams and Turner 2010), male sterility (Feys *et al.* 1994), and in inositol polyphosphates in wound signalling (Mosblech *et al.* 2011). Tomato and Arabidopsis have a single copy of the *COI1* gene each, but other plant species have more than one homologue, such as rice with three homologues (*OsCOI1a*, *OsCOIb*, *OsCOI2*) (Lee *et al.* 2015). The tomato SlCOI1 share more than 68% sequence identity with Arabidopsis counterpart but show less than 57% sequence similarity with rice SOCI1 (Li *et al.* 2004). To date, the physiological role of COI1 in tomato is remained to be determined. In *Solanum nigrum*, *COI1* involved in jasmonate metabolism and systematic protection against insect attacks (VanDoorn *et al.* 2011). In tomato, COI1 mediate signal perception JA is pivotal for steroidal glycoalkaloids (SGA) via a JA-responsive *ETHYLENE RESPONSE FACTOR (ERF)* transcription factor (*JRE4*) (Abdelkareem *et al.* 2017). However, in rice, *OsCOI1a* regulates a basic helix–loop–helix (bHLH) transcription factor 148 expression by SCF^{COI1} complex, leading to enhance tolerance to drought stress (Seo *et al.* 2011), but *OsCOI1b* resulted in leaf yellowing (Lee *et al.* 2015).

Tomato is a typical model plant for studying genetics and developmental physiology because of its small genome and short life cycle (Meissner *et al.* 2002). However, little is known about the molecular characteristics of COI proteins in tomato. Here, a comprehensive bioinformatic analysis of the COI gene family in the whole genome of tomato was performed. A total of nine putative genes encoding COI proteins were identified in tomato. The results of this study display the bioinformatic characteristics of COI family proteins in tomato (SlCOIs), phylogeny, subcellular localization, and tissue-specific expression profile. We also investigated abiotic stress-induced expression analysis. Our data provide a handy reference for the functional analysis of the COI family in tomato.

Materials and methods

Tomato COI genes discovery and physicochemical characteristics

The Arabidopsis COI protein sequences were retrieved from TAIR database (https://www.arabidopsis.org/) (Reiser *et al.* 2017) and used as a query against the Solanaceae Genomics Network (SGN, https://www.solgenomics.net/) (Fernandez-Pozo *et al.* 2015) for tomato with default parameters. Moreover, the COI Pfam domain pattern was retrieved from the Pfam database (Finn *et al.* 2008). The COI proteins of tomato (SlCOIs) were predicted using a hidden Markov model (HMM) profile retrieved from the Pfam database (Finn *et al.* 2008). The *S. lycopersicum* COI protein sequences were searched using the HMMSEARCH program (Finn *et al.* 2011). All redundant COI sequences were excluded and validated whether candidate members of genes processes the COI domain in SMART (Schultz *et al.* 1998) and NCBI CDD (Marchler-Bauer *et al.* 2017). The characteristics of COI genes, including molecular weight (MW, kDa), the grand average of hydropathy (GRAVY), and theoretical isoelectric point (pI), were calculated using the sequence manipulation suite (SMS, http://www.bioinformatics.org/sms2/index.html) (Stothard 2000). All the genes were named in their chronological order on the chromosomes.

Subcellular location prediction of selected SlCOIs

WoLFPSORT program (https://wolfpsort.hgc.jp/) (Horton *et al.* 2007) was used to predict the *in silico* subcellular location of SlCOI proteins. To validate *in silico* predicted subcellular localization of selected SlCOIs, the full-length nucleotide sequences of *SlCOI1*, *SlCOI2*, *SlCOI3* and *SlCOI8* were cloned in frame with green fluorescent protein (GFP) under control of 35S in pMS4::GFP vector. Empty vector (control) and all SlCOI–GFP constructs were transformed into agrobacterium leaves as described by Li (2011). The green fluorescence was observed through the laser scanning confocal microscope (Leica TCS SP8, Germany) after infected for 3 days.

Phylogenetic analysis and gene duplication of SlCOI family members

To investigate the phylogenetic relationship of tomato COI protein with other plant species, the protein sequences of *Physcomitrella patens* (Pp), *Arabidopsis thaliana* (At), *Populus trichocarpa* (Pt), *Sorghum bicolor* (Sb), *Oryza sativa* (Os), *Zea mays* (Zm), *Brachypodium distachyon* (Bd), *Selaginella moellendorffii* (Sm), *Hordeum vulgare* L. (Hv), and *Triticum urartu* (Tu) were acquired from phytozome (https://phytozome.jgi.doe.gov/

pz/portal.html) (Goodstein *et al.* 2012). An unrooted neighbour-joining (NJ) (Saitou and Nei 1987) dendrogram was constructed in MEGAX (Kumar *et al.* 2018) with bootstrap set at 1000 replicates. MCScanX program (https://github.com/wyp1125/MCScanX) was used to predict SlCOI gene duplication events in the tomato genome. The nonsynonymous (K_a), synonymous (K_s) nucleotide substitution rates, and the K_a/K_s ratios were predicted using k-estimator (http://en.bio-soft.net/format/KEstimator.html) (Comeron 1999). The divergence time (T, millions year ago (mya)) was calculated as follows: $T = K_s/2y$ ($y = 1.5 \times 10^{-8}$) (Koch *et al.* 2000).

Gene structure analysis, conserved motif scan, and cis-regulatory motif prediction

The number and distribution of exon–intron in SlCOIs were visualised by submitting nucleotide coding sequences and corresponding genomic sequences to Gene Structure Display Server 2.0 (http://gsds.gao-lab.org/Gsds_about.php) (Hu *et al.* 2015). MEME suite (https://meme-suite.org/meme/tools/meme) (Bailey and Elkan 1994) was used to scan conserved motifs in COI proteins with default parameters. A 1-kb long 5′UTR nucleotide sequence from the start codon was extracted for each SlCOI gene from SNG and submitted in the PlantCARE database (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) (Lescot *et al.* 2002) for *cis*-regulatory elements prediction.

Plant material, abiotic stress and phytohormone treatment

The tomato plant cultivar Micro-Tom was grown in a greenhouse under control conditions: 14 h light / 12 h dark photoperiod, at 25°C / 20°C day/night temperature with relative humidity between 70% and 80%, and photon density of about 120 μmol photons $m^{-2}s^{-1}$. Six-week-old tomato seedling were used for tissue/organ-specific expression and abiotic stress treatments. For tissue/organ-specific expression, various plant parts including, root, shoot, leaves, flower bud, fully opened flower, and fruit at various developmental stages were harvested. The fruit development stages include 1 cm, 2 cm, 3 cm, mature green (MG), breaker (B), and 10-days breaker (B+10) fruits. All the samples were collected in triplicate and mixed thoroughly.

For salinity, drought and phytohormone stresses, 6-week-old tomato seedlings were treated with 200 mM NaCl, cold (4°C), 4% PEG and 0.01 mM ABA, GA3, SA, MeJA, and IAA following Waseem *et al.* (2018). The control plants were treated with fresh water. Seedlings were harvest at 0h, 3h, 6h, 12h and 24h after treatment. Three independent biological replicates were collected, and six seedlings were used for each treatment. All the samples were immediately frozen in liquid nitrogen and stored at −80°C until further analysis.

cDNA preparation and reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR) analysis

Total RNA was extracted from collected samples using TRIZOL reagent following the manufacture's protocol. The RNA was quantified using nanodrop (Thermo USA), and quality was determined through 2% (w/v) stained agarose gel electrophoresis. The first complementary DNA strand was synthesised using Prime Script RT reagent Kit with gDNA Eraser (Takara, Japan). SYBR-Premix Ex Taq-II (TliRNaseH Plus) on CFX96 touch real-time PCR detection system (Bio-Rad, USA) was used to conduct RT-qPCR. The relative expression was normalized using the housekeeping gene of *SlUBQ* (*Solyc01g056940*). We first calculated the amplification efficiency of all the primer pairs analysed in this study (figure 1 in electronic supplementary material at http://www.ias.ac.in/jgenet/). Finally, the relative expression was calculated following $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001).

Statistical analysis

A Student's *t*-test was used to determine the statistical significance of transcript levels between treatments and their corresponding controls. Differential expression data were statistically significant and were marked by a *P*-value of $*P < 0.05$, $** P < 0.01$, and $*** P < 0.001$.

Results

Characteristics of tomato COI gene family

In this study, we used Arabidopsis amino acid sequences (AtCOIs) for BLASTP and HMM searches to screen COI members in the genome of tomato. Further, the presence of conserved COI characteristic F-box domain was confirmed by NCBI CDD-Search and SMART. These nine *SlCOI* genes were subsequently named as *SlCOI1* to *SlCOI9* according to their genomic locus. Detailed physicochemical analysis of putative SlCOI proteins revealed that proteins peptide length and predicted MW ranged widely from 421 (aa)/74.12 kDa (*SlCOI6*) to 675 (aa)/73.78 kDa (*SlCOI9*). The pI ranged from 4.9 (*SlCOI6*) to 7.76 (*SlCOI1*). It was found that some *COI* proteins include *SlCOI2*, *SlCOI3*, *SlCOI4*, *SlCOI6* and *SlCOI8* was hydrophilic due to negative GRAVY values, while others were hydrophobic. *In silico* subcellular localization prediction revealed that *SlCOIs* were localized in three organelles: nucleus, cytoplasm, and chloroplast. *SlCOI1*, *SlCOI2*, *SlCOI3*, *SlCOI6* and *SlCOI8* were in the nucleus; *SlCOI4* and *SlCOI9* were found in the cytoplasm; *SlCOI5* and *SlCOI7* were in the chloroplast (table 1). The tomato *SlCOI* proteins were distributed in seven chromosomes. *SlCOI1*, *SlCOI2*, *SlCOI3*, *SlCOI4*, *SlCOI8* and *SlCOI9* were scattered on chromosomes 1, 2, 4,

5, 9 and 10, respectively. Three SlCOI: *SlCOI5*, *SlCOI6* and *SlCOI7* was distributed on chromosome 6 (figure 1). Only a single pair of SlCOI genes (*SlCOI5-SlCOI8*) displayed segmental duplication while tandem duplications were absent (figure 1). Based on a substitution rate, the duplication events for (*SlCOI5-SlCOI8*) segmental duplication was estimated to have occurred ~27.8 mya (table 1 in electronic supplementary material at http://www.ias.ac.in/jgenet/).

Phylogeny of SlCOI gene family

To insight into the phylogenetic relationship of tomato *SlCOI* proteins with COIs from different plant species, an unrooted NJ tree revealed that all COI proteins were clustered into eight distinct clades. The tomato COI genes were clustered in five clades with COI proteins from *P. patens* (Pp), *A. thaliana* (At), *P. trichocarpa* (Pt), *S. bicolor* (Sb), *O. sativa* (Os), *Zea mays* (Zm), *B. distachyon* (Bd), *S. moellendorffii* (Sm), *H. vulgare* L. (Hv), and *T. urartu* (Tu) indicating high sequence similarity with COI proteins of these plant species (figure 2). *SlCOI8*, *SlCOI5*, and *SlCOI6* were clustered in group II, *SlCOI1* in III, *SlCOI3* in V, *SlCOI4* in VI, *SlCOI7*, *SlCOI1* and *SlCOI9* in group VII. Similarly, we found that COI proteins from different species were also distributed in different groups. For example, Arabidopsis COI proteins clustered in groups I, II and V but only maize in group VI. Moreover, rice COI proteins grouped in I, II, IV, V, and VI while *P. patens* in group III and VI only (figure 2), suggesting that COI proteins exhibited differences in evolution among different species.

Gene structure, conserved motifs, and cis-regulatory elements in SlCOIs

To understand the evolution of the SlCOI gene family, the numbers and distribution of intron/exon in putative tomato COI genes were analysed using corresponding coding and genomic sequences. It was revealed that the exon number in all members of the COI gene family ranged from three to eight. Five exon and four introns were present in *SlCOI5* and *SlCOI6*, while eight exon and seven introns in *SlCOI1*, *SlCOI7* and *SlCOI9*. We found that the position and number of exons and introns in genes belonging to the same class or subclass were similar. This finding supports the phylogenetic relationship of COI family genes (figure 2 and figure 2 in electronic supplementary material and that exon gain and loss have occurred in the SlCOI gene family. The protein architecture of COI proteins from different plant species, including tomato in the MEME program revealed that most of the COIs possess 10 motifs in groups III, V and VI. *COI* proteins in group I have 10 motifs; II and IV containes eight motifs.

Table 1. Physiochemical feature of SlCOI genes in tomato genome.

Gene ID	Name	aa	MW	GRAVY	pI	Chromosome			Exon number	Subcellular location prediction
						Number	Start	End		
Solyc01g099970	SlCOI1	619	68.58	0.187	7.76	1	81818175	81824371	8	nucl:5,cyto:4,chlo:2,mito:1,plas:1,vacu:1
Solyc02g079190	SlCOI2	587	66.12	− 0.154	6.92	2	38380126	38384863	3	nucl:8.5,cyto_nucl:6,cyto:2.5,chlo:1,mito:1,cysk:1
Solyc04g074980	SlCOI3	623	69.2	− 0.044	5.46	4	58453329	58456206	3	nucl:4,cyto:4,E.R.:3,vacu:2,chlo:1
Solyc05g052620	SlCOI4	603	68.56	− 0.168	6.77	5	61968471	61974545	3	cyto:10,chlo:2,nucl:1,vacu:1
Solyc06g008780	SlCOI5	496	54.98	0.119	7.32	6	2707524	2710489	5	chlo:9,nucl:3,cyto:1,vacu:1
Solyc06g008810	SlCOI6	421	47.12	− 0.039	4.9	6	2751079	2755743	4	nucl:7.5,cyto_nucl:6,cyto:3.5,chlo:2,golg:1
Solyc06g054440	SlCOI7	535	57.36	0.255	7.19	6	33700999	33706694	3	chlo:6,nucl:3,cyto:3,mito:2
Solyc09g074520	SlCOI8	581	65.51	− 0.034	7.05	9	61827972	61831778	8	nucl:12,cyto:1,pero:1
Solyc10g076290	SlCOI9	675	73.78	0.189	7.56	10	58549205	58554411	8	cyto:6,nucl:4,chlo:1,mito:1,pero:1,golg:1

aa, amino acid; MW, molecular weight; pI, isoelectric point; GRAVY, the grand average of hydropathy; nucl, nucleus; cyto, cytoplasm; chlo, chloroplast; mito, mitochondria; plas, plasma membrane; vacu, vacuole; ER, endoplasmic reticulum; pero, peroxisomes; golg, golgi apparatus.

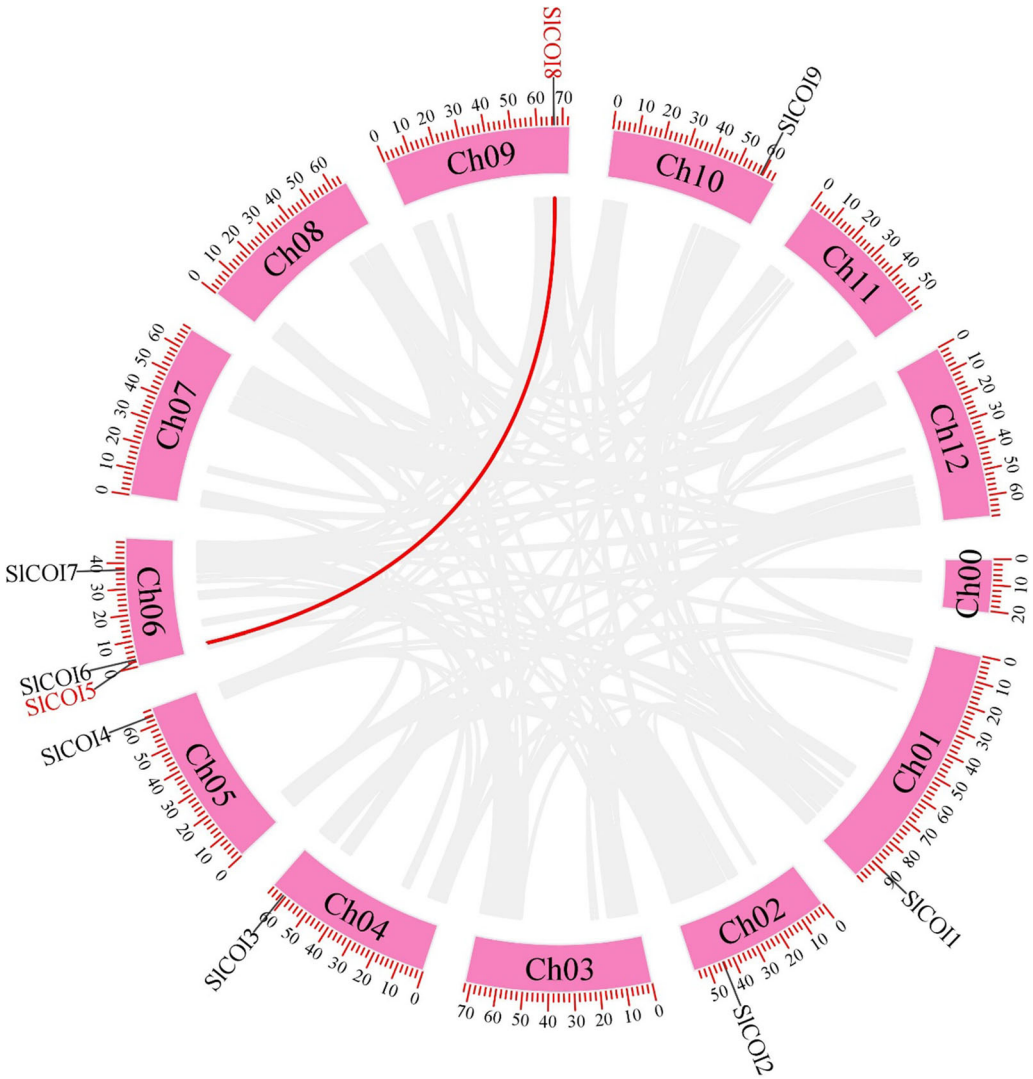

Figure 1. Chromosomal location and synteny plot of nine COI genes in tomato chromosomes. A segmental duplication pair is marked in red.

Moreover, five and six motifs were predicted in groups VIII and VII, respectively (figure 3). In plants, *cis*-regulatory elements play an essential role in regulating gene expression under normal and response to stress stimuli. To study the response of the SLCOI gene to various signal factors, we searched 1 kb sequences upstream of the start codon of the SlCOI family for stress response elements. Figure 3 in electronic supplementary material shows hormone-responsive elements associated with abscisic acid (ABRE), ethylene (ERE), auxin (TGA-element), gibberellins (TATC-box), salicylic acid (TCA-element), and jasmonate (CGTCA/TGACG-motif) were identified in the promoter region of *SlCOIs*. Further, stress-responsive regulatory elements, defence/stress responses (TC-rich repeats), low-temperature responses (LTR), associated with drought inducibility (MBS, MYB), and wound responsive elements (WUN-motif) were identified (figure 3 in electronic supplementary material. These results suggest that the COI family may play a crucial role in the growth and development of tomato and various hormones and stress.

Subcellular localization of SlCOI proteins

Prediction analysis indicated that SlCOIs exhibit various patterns of subcellular localization (table 1). Subcellular localization implied the working position of a protein and is essential for functional gene characterization. To further determined the subcellular localization of SlCOI proteins, GFP fused with selected SlCOI proteins was transiently expressed in tobacco leaf. Consistent with the *in silico* predicted localization using bioinformatics approaches, as shown in figure 4; four COI proteins: SlCOI1, SlCOI2, SlCOI3 and SlCOI8, localized in the nucleus.

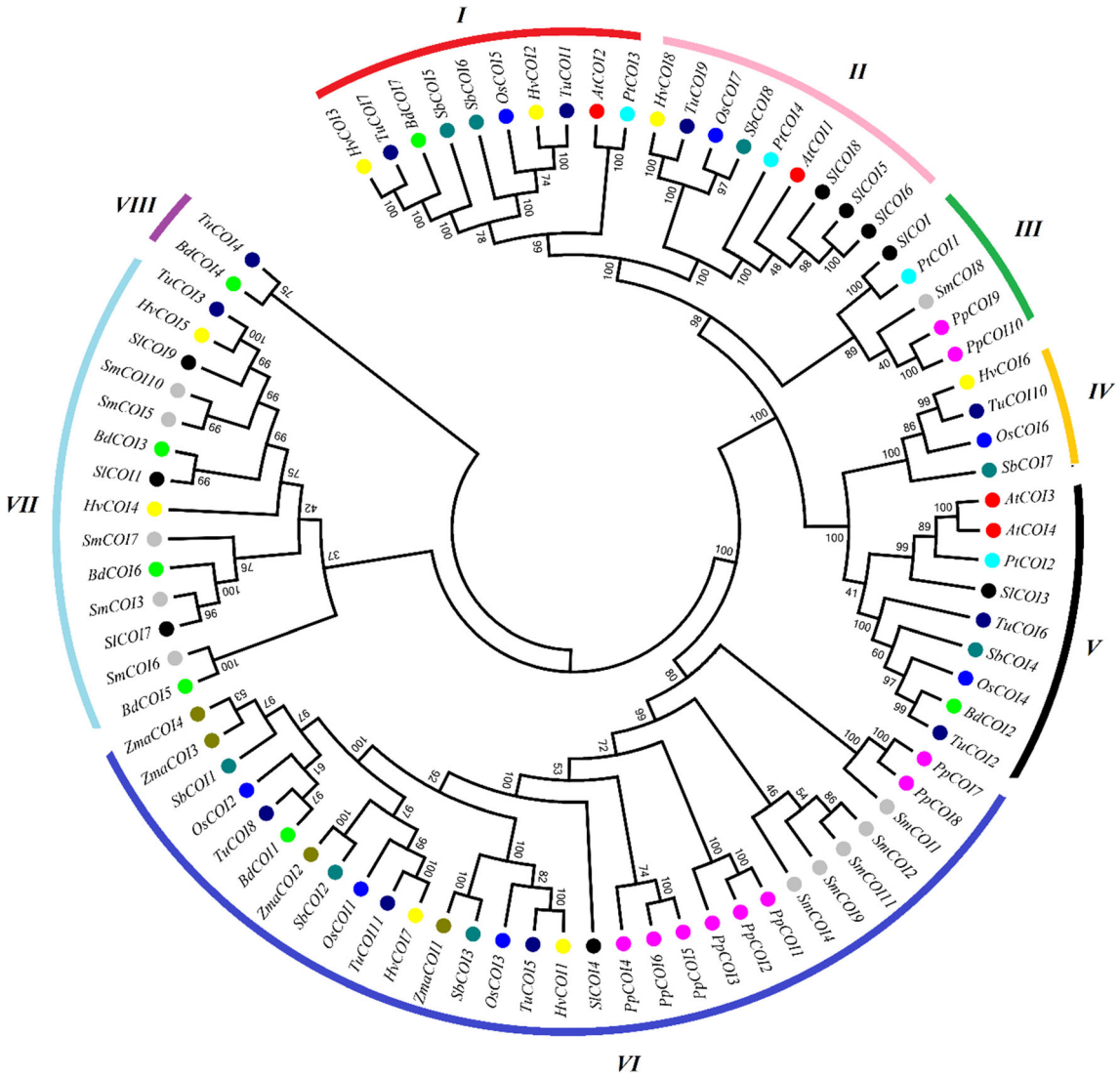

Figure 2. Phylogeny of COI genes. A NJ phylogenetic tree of COI proteins from *Physcomitrella patens* (Pp), *Arabidopsis thaliana* (At), *Populus trichocarpa* (Pt), *Sorghum bicolor* (Sb), *Oryza sativa* (Os), *Zea mays* (Zm), *Brachypodium distachyon* (Bd), *Selaginella moellendorffii* (Sm), *Hordeum vulgare* L. (Hv), and *Triticum urartu* (Tu) including tomato (Sl, *S. lycopercium)* was divided into eight groups (I to VIII). The bootstrap was set at 1000 replicates.

Expression analysis of COI protein family members in different parts of the tomato plant

To elucidate the role of COI protein in plant development, the expression profile analysis in various plant parts, including root, leaf, flower at the bud and fully opened stage and fruits at various developmental stages was investigated (figure 5). It was observed that some genes expressed at relatively high levels in root include *SlCOI1*, *SlCOI3*, *SlCOI4*, *SlCOI7* and *SlCOI9*, indicating that these genes might play a developmental or physiological role in root tissues. Further, some genes had higher transcript abundance at a specific developmental stage concerning fruit development, such as SlCOI expressed specifically in 3 cm fruit, SlCOI4 in fruit at breaker stage, SlCOI6 at 1 cm and *SlCOI8* in 3 cm fruit. Additionally, it was observed that the expression of *SlCOI1*, *SlCOI2*, *SlCOI3* and *SlCOI8* was decreased with the fruit development, and the onset of fruit ripening indicated that these genes might involve in tomato fruit development and ripening (figure 5). Further, we found that promoter sequences of these genes contained ethylene-responsive elements (figure 3 in electronic supplementary material and may involve in ethylene-dependent tomato fruit ripening.

The inducible expression analysis of SlCOIs under salt, drought, and cold

To study the potential roles of SlCOI in abiotic stress responses, we performed expression analysis of SlCOIs under various abiotic stress conditions using quantitative RT-

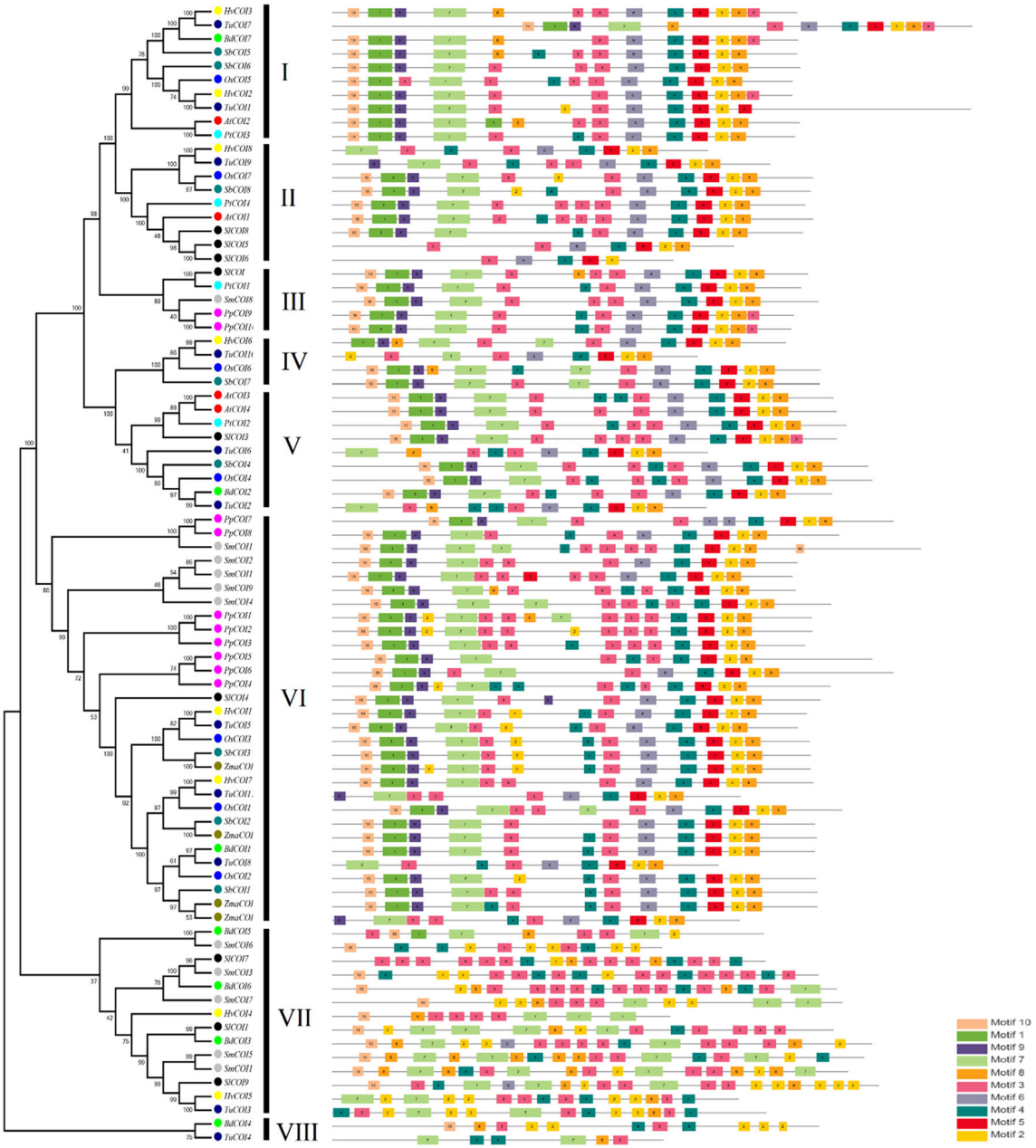

Figure 3. MEME predicted conserved motifs in COI proteins from *Physcomitrella patens* (Pp), *Arabidopsis thaliana* (At), *Populus trichocarpa* (Pt), *Sorghum bicolor* (Sb), *Oryza sativa* (Os), *Zea mays* (Zm), *Brachypodium distachyon* (Bd), *Selaginella moellendorffii* (Sm), *Hordeum vulgare* L. (Hv), and *Triticum urartu* (Tu) including tomato (*Sl; S. lycopercium).* Thus, a total of 10 motifs (1 to 10) were identified in COI proteins.

qPCR. The RT-qPCR data from these stresses at different time intervals (0h, 3h, 6h, 23h, and 24h) is shown in figure 6, demonstrating that SlCOI genes significantly responded to different abiotic stress treatments. For salt stress, the expression abundance of some genes show temporal expression while suppressed for others (figure 6a). For instance, *SlCOI1*, *SlCOI3*, *SlCOI5*, *SlCOI6* and *SlCOI9* were upregulated at all points. *SlCOI4*, *SlCOI8* and *SlCOI7* had similar expression patterns, with the highest expression at 3h and 6h points. *SlCOI2* was upregulated at 6h (figure 6a). *SlCOI1*, *SlCOI3*, *SlCOI6* and *SlCOI8* were induced under drought stress at all point intervals except for *SlCOI1* and *SlCOI8* downregulated at 24h. However, expressions of *SlCOI4*, *SlCOI5*, *SlCOI7*, and *SlCOI9* was upregulated at 3h but showed a gradual decrease in expression until 24h. *SlCOI5* showed strong expression at 6h but downregulated in 12 and 24h (figure 6b). *SlCOI2* was upregulated at all time interval, *SlCOI1*, *SlCOI5* and *SlCOI7* were upregulated at 12 h intervals, but at 6h *SlCOI4* was peak under cold conditions. *SlCOI1* peaked at 12h; *SlCOI3*, *SlCOI8* and *SlCOI9* had peaked expression at 24h (figure 6c). It was observed that *SlCOI6* expressions were peaked at all point against all

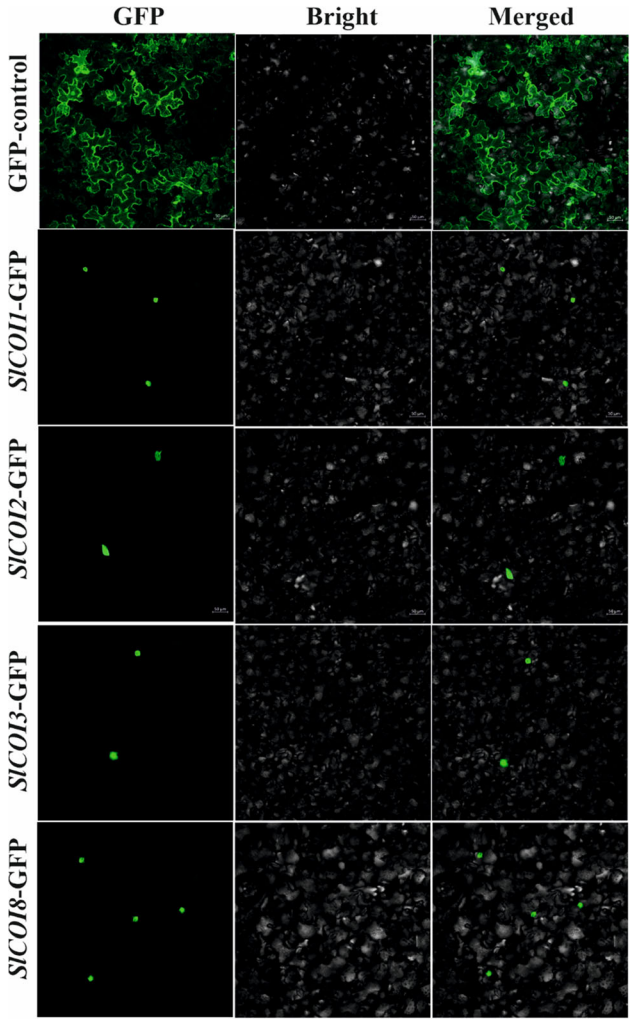

Figure 4. Subcellular localization analysis of SlCOIs proteins. Tobacco leaves transiently expressed SlCOI–GFP fusion proteins that observed through the laser scanning confocal microscope. Scale bars represent 50 μm.

stresses (salt, drought and cold); SlCOI showed similar expression under salt and drought, which were downregulated at 3h and 24h intervals; opposite trends were observed under drought stress. Similarly, *SlCOI9* showed ascending expression under salinity (figure 6a), but descending trends were observed under drought (figure 6b). The extensive involvement of SlCOI genes in response to abiotic stresses implied their potential essential functions.

Phytohormone induced expression profiling of tomato COI genes

The responsiveness of SlCOI genes to different hormones such as ABA, IAA, GA3, SA, and MeJA was investigated by RT-qPCR. For ABA stress, *SlCOI1*, *SlCOI2* and *SlCOI3* upregulated at 12h and 24h intervals; *SlCOI4* and *SlCOI5* upregulated at 24h. *SlCOI8* peaked at 3h, 6h and 12h, while *SlCOI7* had peaked expressions at 6h and 24h. *SlCOI6* was upregulated at 6h but *SlCOI9* peaked at 24h (figure 6d). *SlCOI1*, *SlCOI2* and *SlCOI3* showed peaks at 24h under SA stress. *SlCOI7* decreased with time while *SlCOI6* opposite trends till 12h then downregulated at 24h. *SlCOI5* upregulated at 3h; *SlCOI4* expressions was peaked at 6h; *SlCOI8* and *SlCOI9* upregulated at 3h, and 24h were upregulated (figure 7a). For GA treatment, *SlCOI2*, *SlCOI6* and *SlCOI8* were induced with strong expression at 12h and 24h; *SlCOI7* upregulated at 12h, but *SlCOI4* and *SlCOI5* downregulated. *SlCOI1*, *SlCOI6*, showed temporal expression in all points (figure 7b). *SlCOI2* and *SlCOI8* were upregulated at 24 and 3h points, respectively, against MeJA. *SlCOI1*, *SlCOI5* and *SlCOI7* were upregulated at all point intervals; *SlCOI4*, *SlCOI8*, and *SlCOI9* showed opposite trend (figure 7c). For IAA treatment, most of the genes respond at early point of time. *SlCOI1*, *SlCOI4*, *SlCOI7* and *SlCOI9* were upregulated at all point intervals except at 24h. *SlCOI8* was upregulated at 3h and 12h, suppressed at 24h; *SlCOI6* upregulated at 3h, *SlCOI9* upregulated at 3h but downregulated at later time intervals (figure 7d).

Notably, we found that the expressions of *SlCOI1* increased after treated with all the mentioned hormones used in this study, while the expressions of *SlCOI7* showed a similar pattern between MeJA and IAA treatments but opposite patterns with SA. Expression of *SlCOI9* and *SlCOI8* showed opposite trends between MeJA and GA also between ABA and MeJA treatments, suggested that these hormones may play an antagonistic effect on expressions SlCOIs. Taken together, the variational expression of SlCOI genes under different plant hormone treatment implied that this gene family involved multiple hormonal signals in a complicated way. The detailed role of this gene family in the crosstalk of plant hormones was thus worth studying and may provide us with new insight into the field.

Discussion

The oxylipin (jasmonate and their derivatives) plays a pivotal role in various aspects of plant growth and development and tolerance against abiotic and biotic stresses as including, pigmentation (Zhang *et al.* 2009), root growth and development (Cheng *et al.* 2011), fertility (Turner *et al.* 2002), fruit growth and development (Kondo *et al.* 2000), herbivory (Abuqamar *et al.* 2008), seeds maturation and trichrome formation (Li *et al.* 2004), and leaf yellowing (Reinbothe *et al.* 2009). An F-Box protein, *COI1*, is required for JA mediated responses. COI interacts to form SCF(COI1) E3 ubiquitin ligase complex and trigger jasmonate ZIM-domain (JAZ) proteins for degradation via 26S proteasome (Yan *et al.* 2009).

The availability of genome sequence technology opens new horizons in functional genomics and identifies new genes annotations (Pfeifer *et al.* 2014; Liu *et al.* 2017; Wang *et al.* 2017; Waseem *et al.* 2018). In this sense, the COI gene family has been identified and characterized in various plant

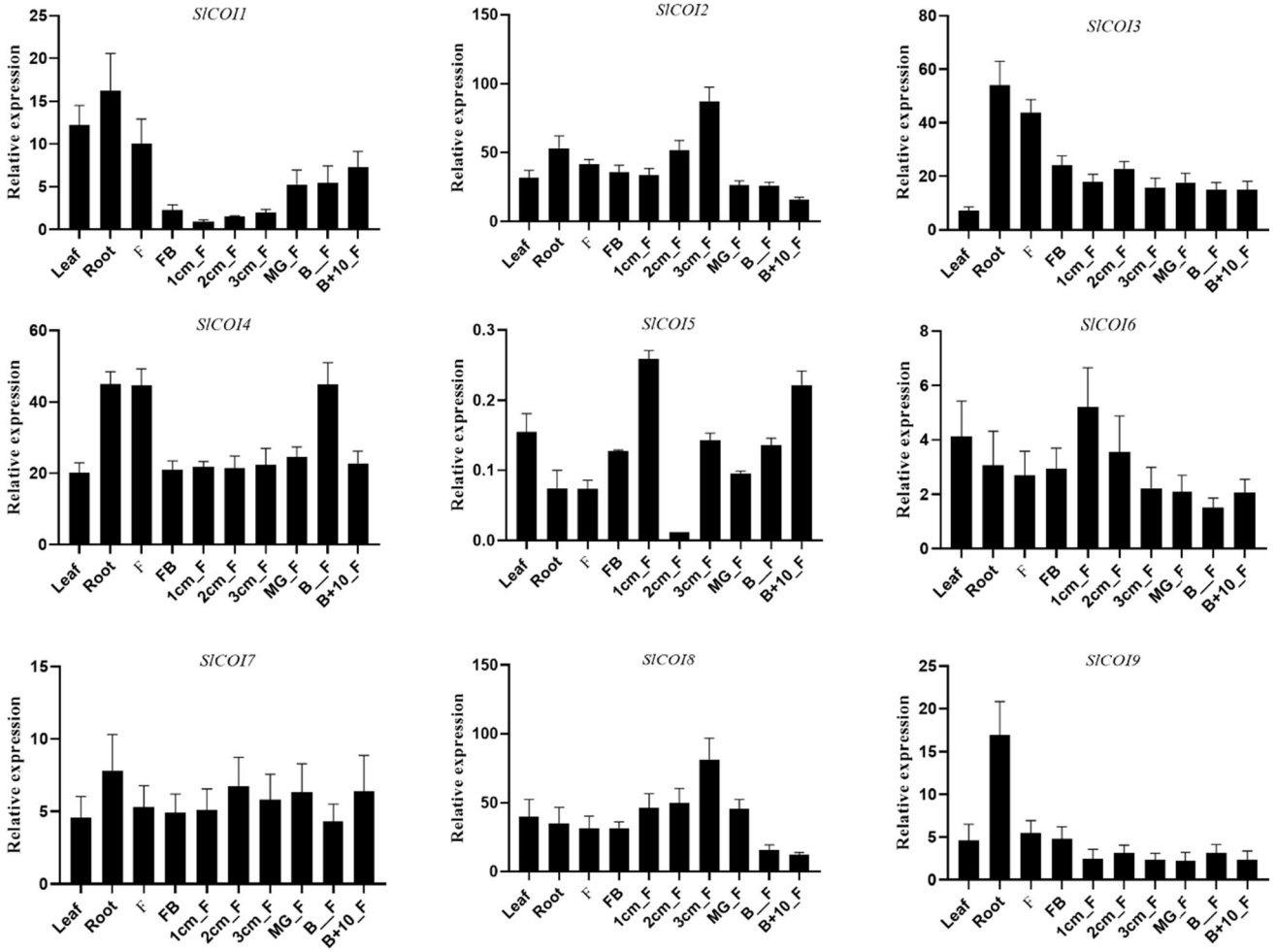

Figure 5. Tissue-specific expression analysis of tomato *Pht1* genes. The RNA was prepared from different tissues including root, FB, flower bud; F, flower; leaves: 1cm_F, 2cm_F, 3cm_F; _F, fruit, MG_F, mature green; B_F, breaker fruit; B+10_F, 10 days breaker fruit. The relative expression levels of each of the tomato COI genes were indicated as a percentage of the constitutive *SlUBQ* expression activity. Each bar was the mean of five biological replications with standard error.

species such as Arabidopsis (Xie *et al.* 1998), rice (Lee *et al.* 2015), *Aquilaria sinensis* (Liao *et al.* 2015), and tobacco (Ishiga *et al.* 2013). In this study, nine tomato COI genes were identified in the whole genome using four Arabidopsis COI sequences as the query. The bioinformatics analysis revealed that *SlCOI*s showed hydrophobic and hydrophilic characteristics and was *in silico* localized in the nucleus, cytoplasm, and chloroplast (table 1). These genes were physically located in seven chromosomes and shared molecular weight as low as 74.14 kDa and as high as 73.78 kDa. The phylogenetic analysis of tomato SlCOI proteins with other plant species revealed that all the *SlCOI* proteins were clustered into five clades (figure 2). It was also observed that *SlCOIs* with similar gene configuration (exons/introns) grouped in the same cluster (figure 2 in electronic supplementary material).

The expression analysis of *SlCOIs* in different tomato plant parts was investigated to examine COI functions (figure 5). It was observed that these proteins show significant expression levels in a specific tissue or plant organ. For example *SlCOI1*, *SlCOI3*, *SlCOI4*, *SlCOI7* and *SlCOI9* was specifically expressed in root tissues with high transcript abundance but *SlCOI6* in 1 cm fruit; *SlCOI8* in 3 cm fruit, and *SlCOI4* in breaker stage fruit. These findings suggested that these genes might play a pivotal role in developing these plant organs. For further insight into the functions of *SlCOIs*, *cis*-regulatory elements (figure 3 in electronic supplementary material) and expression under salt, drought, cold and exogenous phytohormones such as ABA, IAA, GA, MeJA and SA were analysed (figure 2 in electronic supplementary material). The hormone associated *cis*-elements such as ABA, IAA, GA, MeJA and SA, and stress-responsive *cis*-regulatory sequences such as salinity, light, drought, and defence were found in promoters of tomato COI genes. These suggested that *SlCOI* genes involved in different regulatory mechanisms activated under abiotic and biotic stimuli.

The COI genes expression regulated by various plant hormones. For instance, Arabidopsis (*AtCOI1*) involved in JA mediated pollen development (McConn and Browse

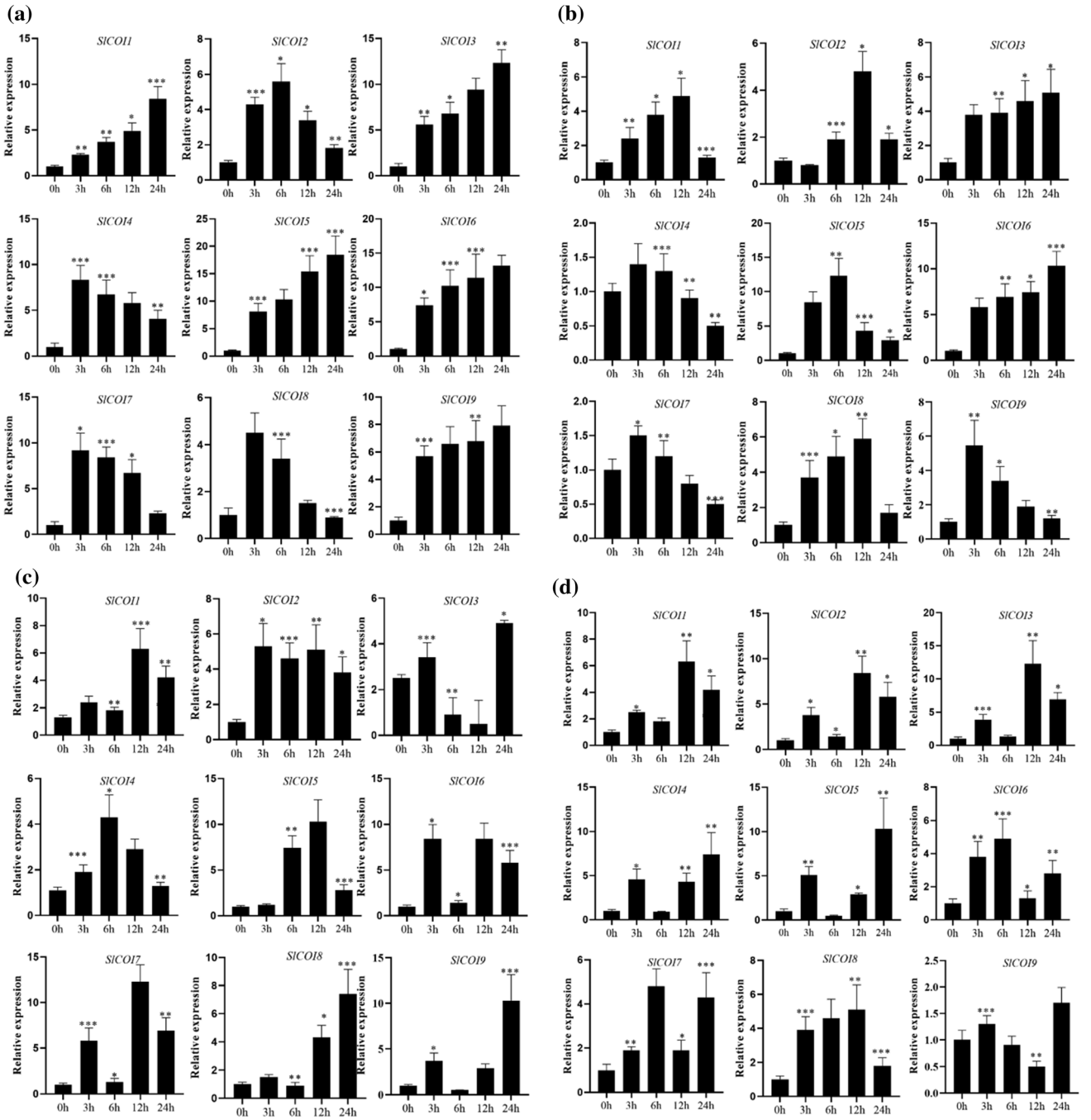

Figure 6. Expression profile of tomato COI genes under (a) 200 mM salt (b) drought (4% PEG), (c) cold (4°C), 0.01 mM, and (d) ABA. Six-week-old tomato seedling was selected for treatments. The fold changes in gene expression were obtained according to the $2^{-\Delta\Delta Ct}$ method. Data are presented as mean and SD. *P* values obtained from pairwise comparisons using student *t*-test are shown (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

1996) and insects defence (Mcconn *et al.* 1997). COI genes were also induced by some abiotic stresses. *TaCOI1* was upregulated under salt and drought stresses, but *TaCOI3*, *TaCOI6* and *TaCOI8* were downregulated (Jian-fang *et al.* 2018). However, tomato COI genes were induced by salt, drought, and cold treatments (figure 6, a–c). *SlCOI1*, *SlCOI3*, *SlCOI5*, and *SlCOI6* were upregulated by salt; *SlCOI3*, *SlCOI6*, and *SlCOI8* induced by drought; *SlCOI6*, *SlCOI7*, *SlCOI8*, and *SlCOI9* were peaked under cold stress. In this study, inducible expression analyses revealed that SlCOI expression could be induced by at least one hormone. In wheat, *TaCOI2* and *TaCOI6* were downregulated by treatment with SA, MeJA, PEG, and IAA (Jian-fang *et al.* 2018). Consistent with this, the expression of SlCOI genes was also at least induced under one exogenous phytohormone (figures 6, d and 7), such as *SlCOI1*, *SlCOI2*, *SlCOI3*,

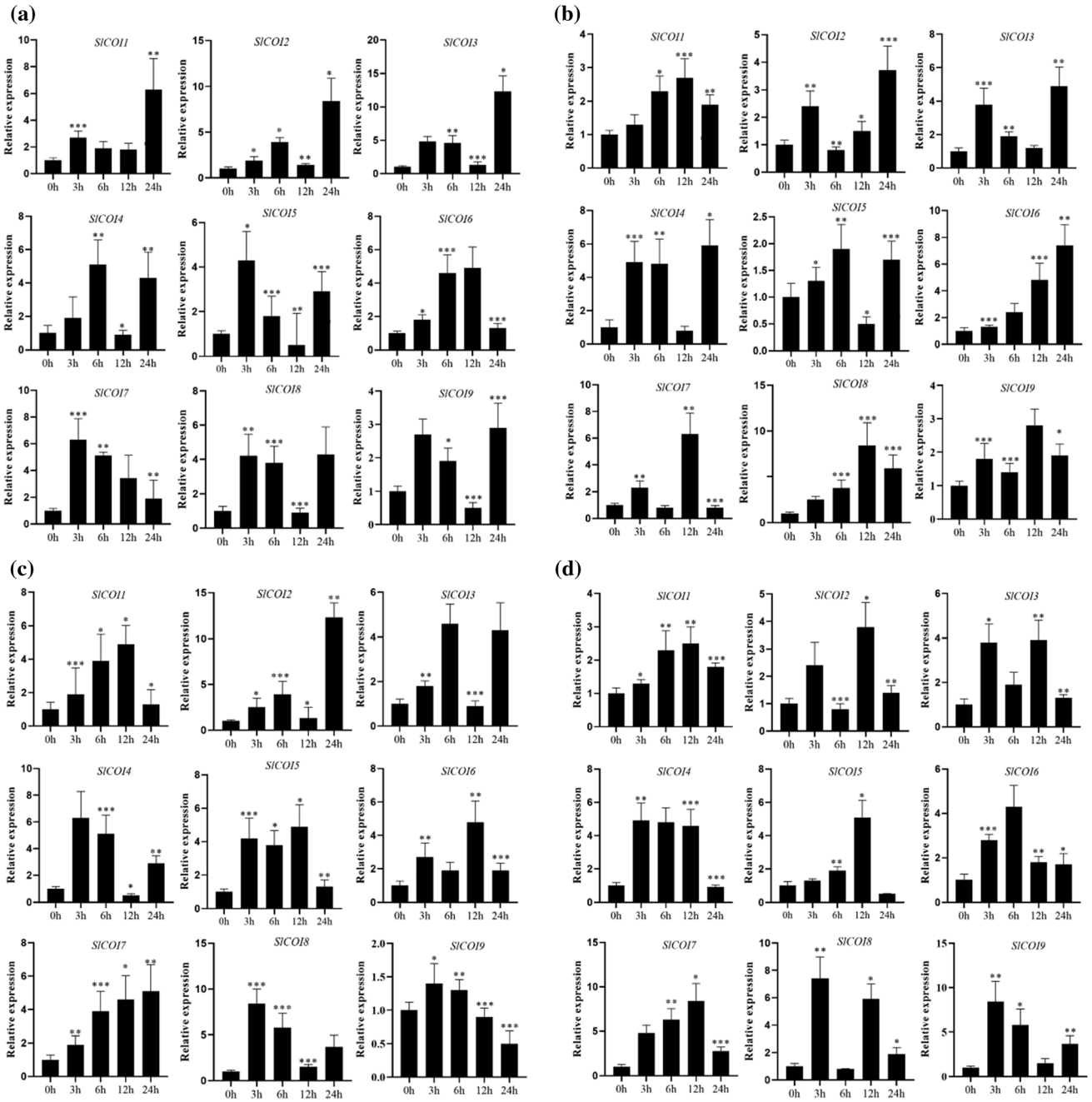

Figure 7. Expression profile of tomato COI genes under (a) SA, (b) GA (c) MeJA, and (d) IAA. Six-week-old tomato seedling was selected for treatments. Relative fold changes in gene expression were obtained according to the $2^{-\Delta\Delta Ct}$ method. Data are presented as mean and SD. In addition, *P* values obtained from pairwise comparisons using student *t*-test are shown (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

SlCOI4, *SlCOI5*, and *SlCOI7* were peaked under ABA, GA, SA and MeJA; *SlCOI6* was upregulated by GA; *SlCOI7*, *SlCOI8*, and *SlCOI9* were upregulated by IAA; *SlCOI5*, *SlCOI7* and *SlCOI4* were downregulated by GA, SA and MeJA. These suggest that tomato COIs might be directly or indirectly associated with enhanced tolerance against these stresses and plant development. Further investigations will need the functional characterization of these genes in the future.

In conclusion, these results provide a comprehensive overview of the COI gene family in tomato model plant, including phylogeny, *in silico* subcellular location analysis, and expression profile analysis in various plant parts under normal conditions. Moreover, the role of *SlCOIs* in response to salinity, drought, cold and exogenous phytohormones such as ABA, IAA, GA, SA, and MeJA provides a more comprehensive understanding of these genes' functions.

Further, functional characterisation of these genes in the future validates the conclusion presented in this study.

Author contributions

MW performed the experiments, MW and IS carried out the analyses and MW, MMA, and IS drafted the manuscript and revised it. All authors approved the final version of the manuscript.

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